FORMULATION AND EVALUATION OF BIODEGRADABLE IMPLANTABLE DRUG DELIVERY SYSTEM OF CLINDAMYCIN HYDROCHLORIDE

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MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

S.BEETHA ROHINI

Reg .No.26106301

Under the Guidance of

Dr.S.UMADEVI

M.Pharm., Ph.D.,

Professor and Head

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DEPARTMENT OF PHARMACEUTICS

RVS COLLEGE OF PHARMACEUTICAL SCIENCES

Sulur, Coimbatore.

CERTIFICATE

This is to certify that the research project work entitled " **Formulation and Evaluation of Biodegradable Implantable Drug Delivery System of Clindamycin Hydrochloride** " is a bonafide work of **Ms.S.BEETHA ROHINI (Reg.No.26106301)** carried out in the **Department of Pharmaceutics, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore**, in Partial fulfilment of the requirements for the award of degree in **Master of Pharmacy in Pharmaceutics** of **The Tamilnadu Dr.M.G.R.Medical University, Chennai** and completed the work under the guidance and supervision of **Dr.S.UMADEVI** during the academic year 2011-2012 This work is original and contributory.

Place : Coimbatore

Date :

Dr.R.VENKATANARAYANAN

M.Pharm.,Ph.D

Principal

R.V.S. College of Pharmaceutical Sciences

Sulur, Coimbatore.

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This is to certify that the research project work entitled " **Formulation and Evaluation of Biodegradable Implantable Drug Delivery System of Clindamycin Hydrochloride**" is a bonafide work of **Ms.S.BEETHA ROHINI (Reg.No.26106301)** carried out in the **Department of Pharmaceutics, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore** in Partial fulfilment of the requirements for the award of degree in **Master of Pharmacy in Pharmaceutics** of **The Tamilnadu Dr.M.G.R.Medical University, Chennai**, under my supervision and guidance to my fullest satisfaction. This work is original and contributory.

Place : Coimbatore

Date :

Dr.S.UMADEVI

M.Pharm.,Ph.D

Professor and Head

Department of Pharmaceutics

R.V.S. College of Pharmaceutical Sciences

Sulur, Coimbatore.

DECLARATION

I hereby declare that this dissertation work entitled " **Formulation and Evaluation of Biodegradable Implantable Drug Delivery System of Clindamycin Hydrochloride**" was carried out by me in the Department of Pharmaceutics at R.V.S.College of Pharmaceutical Sciences under the guidance, of **Dr.S.UMADEVI,** Professor and Head, Department of Pharmaceutics in R.V.S.College of Pharmaceutical Sciences, Sulur, Coimbatore . This work is original and contributory.

Place : Coimbatore **S.BEETHA ROHINI**

Date : (Reg.No.26106301)

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Date: **S.BEETHA ROHINI**

Place: Coimbatore **(Reg.No.26106301)**

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LIST OF ABBREVIATIONS

Abbreviations used	Meaning
PEG	Polyethylene glycol
FT-IR	Fourier transform infrared
DSC	Differential scanning calorimeter
LB	Luria bertani
K_0	Zero order rate constant
K_1	First order rate constant
K_H	Higuchi rate constant
K_{kp}	Koresmeyer-peppas rate constant
K_{HC}	Hixson-Crowell rate constant
MIC	Minimum inhibitory concentration
HPMC	Hydroxy propyl methyl cellulose
MC	Methyl cellulose
EC	Ethyl cellulose

ABSTRACT

The purpose of this study is to formulate implant containing Clindamycin hydrochloride that could be used in the treatment of periodontitis. The implants were formulated by using biodegradable polymer, gelatin and sodium alginate with PEG 400 as a plasticizer. The drug and polymer compatability in implants were studied by FT-IR and DSC. There was no interaction between the drug and polymer. The implant was evaluated for physicochemical properties such as weight uniformity, thickness, content uniformity, %moisture loss and surface pH. The result of physicochemical properties was uniform for all the formulations. The in-vitro results showed that increase in the polymer concentration (70:30) prolong the drug release upto 24hrs. Optimized formulation F7 release 84.90% of drug at the end of 24th hr and considered as a best formulation. The release mechanism for invitro release was studied by using various mathematical models. The 'n' value for the koresmeyer-peppas equation was in the range of 0.87-0.98 indicating the anamolous behaviour (non-fickian release). In-vitro antibacterial activity was carried out in *Staphylococcus aureus* and *Enterobacter aerogen* had an inhibitory effect after 24hrs of incubation. The stability studies were carried out at room temperature, dark place and direct sunlight which does not shows any significant change after one month.

1. INTRODUCTION

Novel drug delivery system (NDDS) has gained a considerable attention from the last few decades. In the form of NDDS, an existing drug molecule can get a new life, thereby increasing the market value, competitiveness and novelty in drug delivery. Implantable drug delivery system (IDDS) is an example of such systems available for therapeutic use.

Historically the subcutaneous implantation of drug pellets is known to be the first biomedical approach aiming to achieve the prolonged and continuous administration of drugs. This first generation of IDDS was produced by compressing drug crystals, with or without a small fraction of pharmaceutical excipients into tiny, cylindrical solid pellets that could be readily implanted into subcutaneous tissue[1].

In 1861 Lafarge first introduced the concept of implantable systems for sustained drug administration. The concept was later used to produce solid implants containing steroid hormones-initiating the use of pure drug with no added excipient. Sterile tablets consisting of the highly purified drug, usually compressed without excipient intended for subcutaneous implantation in body tissue. The device thus prepared had a high degree of hardness and virtually zero porosity. Since water did not penetrate the matrix drug release occurred principally by surface dissolution. Due to the inherent poor solubility of steroid drugs this method provided a good form of depot medication[2].

The implantable therapeutic systems are mainly approached for

- Long term
- Continuous drug administration and
- Controlled release

Over the years a number of approaches have been developed to achieve the controlled administration of biologically active agents via implantation (or insertion) in tissues. These approaches are outlined as follows[1].

I. Controlled drug delivery by diffusion process
   A. Polymer membrane permeation controlled systems containing
      1. Non porous membrane
      2. Microporous membrane
      3. Semipermeable membrane

   B. Matrix diffusion controlled systems containing
      1. Lipophilic polymers
      2. Hydrophilic swellable polymers

3. Porous polymers

C. Microreservoir dissolution controlled systems containing
   1. Hydrophilic reservoir/ lipophilic matrix
   2. Lipophilic reservoir/ hydrophilic matrix

II. Controlled drug delivery by activation process
   A. Osmotic pressure activated
   B. Vapour pressure activated
   C. Magnetically activated
   D. Phonophoresis activated
   E. Hydration activated
   F. Hydrolysis activated

III. Controlled drug delivery by feedback regulated process
   A. Bioerosion regulated drug delivery
   B. Bioresponsive drug delivery

Most of the approaches listed above can be adopted to fabricate the systems for controlled release of biologically active agents. Polymers of both non-biodegradable and biodegradable types can be used depending on the requirement.

Biodegradable polymers:

Biodegradable systems have gained much popularity over non-degradable delivery system, as they are eventually absorbed or metabolized and excreted by the body. This alleviates the need for surgical removal of the implant after the conclusion of therapy increasing patient compliance.

Non-biodegradable polymers:

These are inert in the environment use and serve essentially as a rate limiting barrier to the transport and release of drug from the device. The major disadvantage is the implants have to be removed surgically once the conclusion of the therapy from the site of implantation since they are non- biodegradable.

1.1 Polymers used in the implants[3]

Carbochain polymers

Polyethylene

Polypropylene

Polytetrafluoroethylene

Poly-α-cyanacrylates

Heterochain polymers

Poly (DL-lactide co-glycolide)

Polyurethanes

Polylactic acid

Polyorthoester

Collagen

Gellan

Gelatin

Mucopolysaccharides

1.2 Ideal requirements of implantable drug delivery systems[4]

- Environmentally stable
- Biocompatible
- Sterile
- Biostable
- Improve patient compliance by reducing the frequency of drug administration over the entire period of treatment.
- Release the drug in a rate-controlled manner that leads to enhanced effectiveness and reduction in side effects.
- Readily retrievable by medical personnel to terminate medication.
- Easy to manufacture and relatively inexpensive.

1.3 Advantages of the implantable drug delivery system[4]

- Improved efficiency
- Very effective
- Small dose is sufficient to elicit the action
- Reduced side effects
- On-spot delivery
- Convenient therapy
- Plasma drug levels are continuously maintained in a therapeutically desirable range

- Harmful side effects from systemic administration can be reduced or eliminated by local administration from a controlled release system.
- Continuous small amounts of drug may be less painful than several large doses.
- Patient compliance is improved.

1.4 MECHANISM OF DRUG RELEASE FROM IMPLANTABLE DEVICES[2]

Drug release from most implantable devices is controlled by any one of six different mechanisms discussed below.

1.4.1 Diffusion controlled

These devices are based on Fick's law of diffusion which states that the rate of transfer of a diffusing substance through unit area of a section. In this case the rate of release is controlled by diffusion of drug through a polymeric membrane. Diffusion-controlled devices can be further classified into membrane-permeation controlled, matrix-controlled and micro reservoir-dissolution controlled.

❖ **Membrane-permeation controlled**

In membrane-permeation controlled devices the drug reservoir is surrounded by a membrane and because of the presence of the two distinct drug-reservoir and membrane phases these are known as heterogeneous devices. When the device containing a highly hydrophilic drug is placed in aqueous dissolution medium, water penetrates the coating and dissolves the drug and the concentrated drug solution diffuse out through the polymeric membrane. The release rate of the drug is controlled by the diffusion rate of drug solution through a spherical membrane permeation controlled system with saturated reservoir is given by equation.

$$\frac{dM}{dt} = \frac{4\pi Dk(C1 - C2)ab}{b - a}$$

Where, $\frac{dM}{dt}$ is related to the drug concentration in the matrix and the rate of polymer erosion

D is the diffusivity of drug unit thickness of polymer

k is the partition coefficient (ratio of solubility of drug in the polymer divided by the solubility of drug in the surrounding medium) of drug across the polymer membrane

C1 is the concentration of drug inside the sphere

C2 is the concentration of drug in the surroundings

a is the inner radius of the coat and

b is the outer radius of the coat.

❖ **Matrix controlled**

In matrix-controlled devices the drug is uniformly distributed throughout the polymer and hence these are known as homogenous devices. In the presence of dissolution medium drug at the surface dissolves first and is released in the dissolution medium. In many cases the dissolved drug creates a depletion boundary separating the empty or drug-depleted polymer from the drug loaded polymer matrix. The drug release rate is controlled by the diffusivity barrier provided by at the empty matrixes which increase in thickness with time. For a matrix system that is exposed to the dissolution medium on all the sides the surface area of the inward-moving depletion boundary decrease resulting in a decrease in drug release rate which depends on the device geometry.

❖ **Micro reservoir dissolution-controlled**

In these devices the drug reservoir is made of a suspension of solid drug particles in an aqueous solution of a water miscible polymer forming millions of microscopic drug reservoirs in a polymer matrix the device is coated with a rate-controlling membrane to further modify the drug release.

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1.4.2 Chemically controlled

Chemically controlled drug delivery systems regulate the drug release rate by a chemical reaction with the polymer. The principal advantage is that in contrast to a nonbioerodible system the polymer is dissolved and absorbed by the body. However the fate of these polymeric products in the body must be carefully observed and rigorous testing is required to confirm the safety of the polymer. The two predominant mechanisms for chemically controlling drug release are bioerosion and pendent chain.

❖ **Bioerosion**

The bioerosion or biodegradation systems involve breakdown of the polymer into small water soluble molecules. Bioerosion-controlled devices are matrix controlled with uniform drug distribution inside the polymer. As the polymer is broken down water comes in contact with the drug leading to its dissolution and release. Depending on water-soluble components water may penetrate throughout the device or come in contact only with the surface. In the former case polymer erosion starts throughout the matrix: these devices are

known as bulk eroding. On the other hand if the polymer is hydrophobic and water does not penetrate inside the device, erosion only on the surface; these devices can be called surface eroding. The drug rate from a surface eroding polymeric matrix with uniform drug distribution is given by eq.

$$\frac{dM}{dt} = ks$$

Where, K is a constant,

$\frac{dM}{dt}$ is related to the drug concentration in the matrix and the rate of polymer erosion

S is the surface area of the system.

❖ **Pendent chain**

The other mechanism for chemically controlled release of drug is known as the pendent-chain system where the drug is attached to the polymer backbone by a labile chemical linkage. In the presence of water or enzymes the labile linkage breaks the drug. The pendent chain may be water soluble or insoluble; a water backbone may serve as a drug carrier to a specific cell or organ where the drug is released by metabolism. Insoluble pendent chains serve as a depot from which the drug slowly released.

1.4.3 Solvent activated

Solvent-activated system release active agents because of controlled penetration of a solvent into the device; they can be controlled by swelling or osmotic pressure.

❖ **Swelling controlled**

Swelling- controlled systems are similar to matrix-type devices except that the dispersed drug is immobilized inside a glassy polymer and therefore there is no diffusion of drug. When this device is placed in water the outer polymer region begins to swell, resulting in relaxation of the polymer chains. This allows the locked drug to diffuse outward. Therefore two fronts are observed: one moving inward, separating the polymer in the glassy state from the rubbery state and the second moving outward separating the swollen rubbery polymer from the surrounding aqueous medium. The drug release is determined by the rate relaxation of the chains that unlock the drug.

❖ **Osmotically controlled**

In osmotically controlled system an osmotically active agent such as water soluble salt is placed inside a rigid semi permeable housing, which is separated from the drug compartment by a movable partition. The semi permeable housing draws water inside by osmosis, leading to an increase in volume and exertion of pressure on the movable partition. The partition, in turn pushes the drug out of the compartment through a delivery orifice or cannula. Thus, the drug delivery rate is controlled by the mass movement of water across the permeable membrane.

1.4.4 Externally regulated

These systems have the important advantage that the drug-delivery rate can be externally increased on demand ever after the device has been implanted. Four predominant techniques have been evaluated with externally modulated implant: magnetically controlled, ultrasonically activated, thermally activated and electrically controlled.

- **Magnetically controlled**

In magnetically controlled drug-delivery systems the drug and magnetic beads are uniformly dispersed inside semi elastic polymer matrix made of a nonbiodegradable polymer such as ethylene-vinyl acetate copolymer (EV Ac). When the device is placed in dissolution medium the drug release follow matrix diffusion control. However, when the device is placed in a magnetic field, the magnetic beads attempt to a align with the applied magnetic field including a torque on the magnet and a slight rearrangement of the polymer. In an oscillating magnetic field, the beads tend to oscillate compressing and expanding the polymer in the process.

- **Ultrasonically activated**

In these systems the drug is uniformly distributed inside a polymer and an external ultrasonic field is applied to activate drug release. They have been evaluated for both non biodegradable polymers (EVAc) and biodegradable polymers [polyesters, polynanhydrides, polyglycolides, polylactides and sebacic acid]. In the case of biodegradable polymers application of ultrasound increases the drug release as well as the polymer degradation rate. In both the biodegradable and nonbiodegradable polymer systems the drug release rate was controlled by the intensity, frequency and duration of the ultrasound.

- **Thermally activated**

A series of thermosensitive hydrogels that show significant swelling changes in water in response to temperature have been prepared and evaluated. These polymers responded to

temperature change based on the Flory-Huggins theory that a change in temperature affects hydrogen bonding which in turn, affects swelling. A linear correlation is observed between the diffusion coefficient for entrapped drug and polymer swelling.

❖ **Electrically controlled**

Electrically controlled systems provide drug release by the action of an applied electric field on a rate-limiting barrier membrance or a solute thus modulating its transport across it. Grimshaw reported four different mechanisms for the transport of proteins and neutral solutes across hydrogel membranes:

- Electrically and chemically induced swelling of a membrane to alter the effective pore size and permeability
- Electrophoretic augmentation of solute flux within a membrane
- Electroosmotic augmentation of solute flux within the membrane and
- Electrostatic partitioning of charged solutes into charged membrane.

1.4.5 Self-regulated

These are biofeedback-controlled system, where the drug release rate is dependant on the body's need for the drug at a given time. From a therapeutic viewpoint these systems may come close to duplicating the release from a gland such as the pancreas. A variety of mechanisms have been employed to obtain self-regulated delivery.

❖ **Ionic strength and pH responsive**

These devices take advantage of the fact that polymers containing weakly acidic or basic side groups develop a charge in alkaline or acidic pH respectively. In a cross-linked water-insoluble polymer, this results in water uptake and corresponding swelling of the polymeric membrane with opening of molecular pores and increased drug release rate.

❖ **Glucose responsive**

Glucose Oxidase catalyses a reaction between glucose and oxygen in the body fluids to form gluconic acid, which reduces the pH of the microenvironment. The insulin-release systems based on glucose Oxidase utilize this drop in pH to trigger an increased release.

❖ **Urea responsive**

The device of disk containing hydrocortisone incorporated into a biodegradable polymer with pH dependent degradation. This disk is coated with a hydrogen containing immobilized urease. In physiological- buffer base line hydrocortisone is released due to the hydrolysis of the polymer and diffusion of drug. In the prevalence of urea the enzyme urease increases the pH of microenvironment by converting urea into ammonium bicarbonate and

ammonium hydroxide.This increase in pH results in results in increased hydrolysis of the biodegradable polymer and increased hydrocortisone release.

1.5 NON-DEGRADABLE AND BIODEGRADABLE IMPLANT SYSTEMS

1.5.1 Non-degradable implant systems

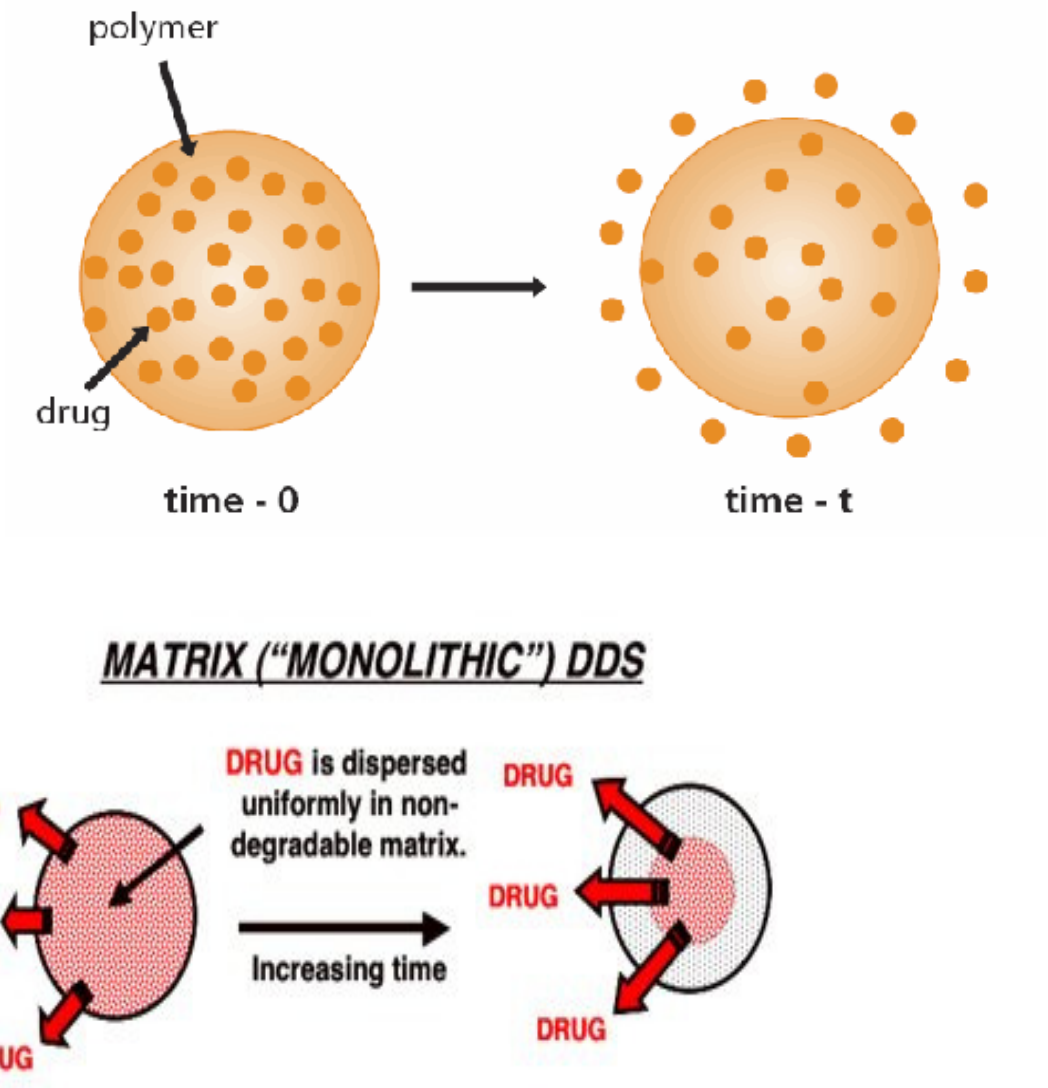
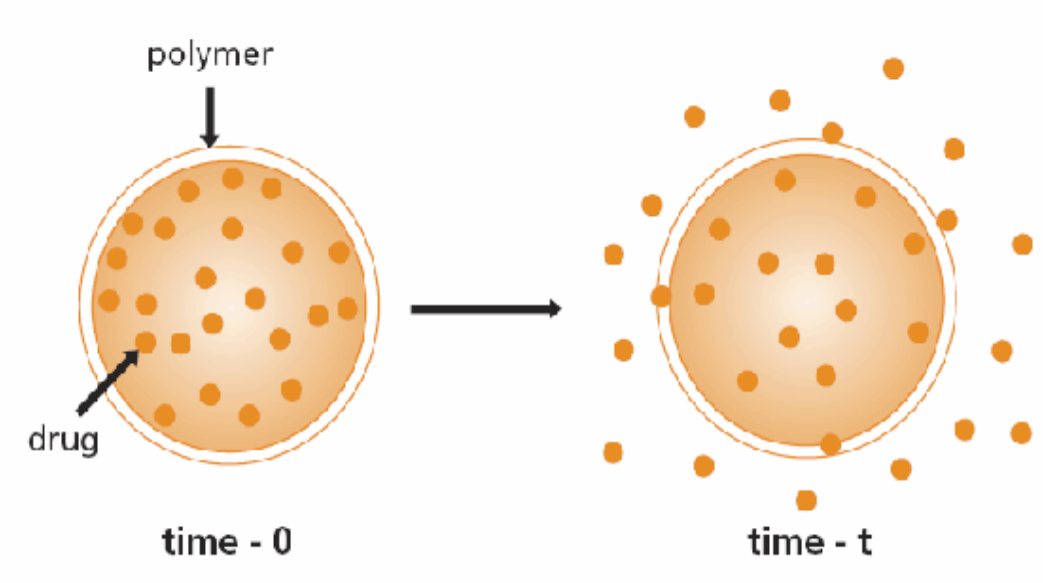


Figure 1.1: Matrix system showing diffusion of the drug across the polymer

There are several types of nondegradable implantable drug delivery systems available in the market place today, but the nondegradable matrix system and reservoir systems are the two most common forms.

In the polymeric matrix system, the drug is dispersed homogeneously, inside the matrix material. Slow diffusion of the drug through the polymeric matrix material provides sustained release of the drug from the delivery system.



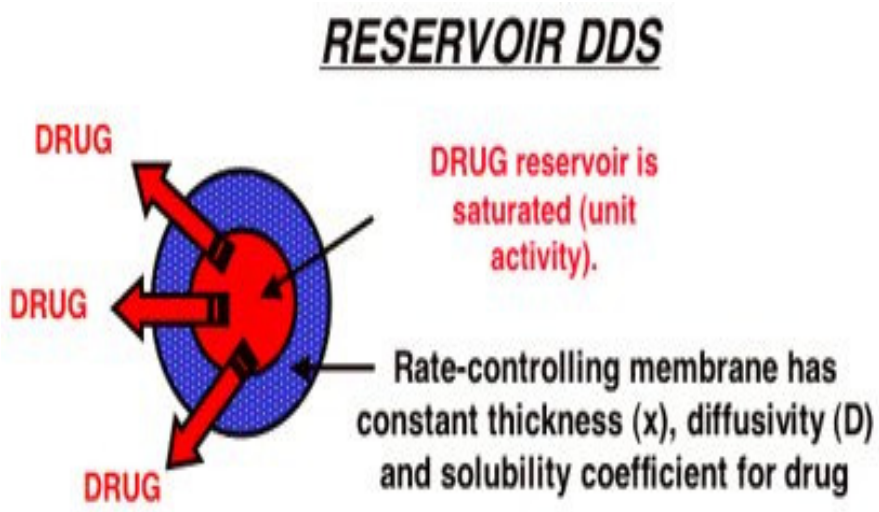


Figure 1.2: Reservoir systems showing diffusion of the drug across the polymer

The reservoir-type consists of a compact drug core surrounded by a permeable nondegradable membrane whose thickness and permeability properties can control the diffusion of the drug into the body[5]. The release kinetics of drug from this system suggest that if the concentration of the drug within the reservoir is in constant equilibrium with the inner surface of the enclosed membrane, the driving force for diffusional release of the agent is constant and zero-order release kinetics of the drug from the delivery system is obtained. This system is nondegradable.

1.5.2 Bio degradable implant systems

Biodegradable systems have gained much popularity over nondegradable delivery systems. The major advantages of biodegradable systems include the fact that the inert polymers, used for the fabrication of the delivery system, are eventually absorbed or excreted by the body. This alleviates the need for surgical removal of the implant after the conclusion of therapy thereby increasing patient acceptance and compliance[6].

However, developing biodegradable systems is a more complicated task than formulating nondegradable systems. When fabricating new biodegradable systems, many variables must be taken into consideration. For instance, the degradation kinetics of the polymer, in vivo, must remain at a constant rate to maintain sustained release of the drug. Many factors can affect the rate of degradation of the polymer in the body. Alterations in body pH or temperature can cause a transient increase or decrease in the degradation rate of the system. The surface area of the delivery system also plays an important role in its degradation[7]. As the system is eroded, the surface area of the implantable system decreases.

1.6 THERAPEUTIC APPLICATIONS[2]

- **Ocular disease**

Ocusert containing pilocarpine base and alginic acid in a drug reservoir surrounded by a release-rate controlling ethylene-viny1 acetate membrane. The ocusert system provides an initial burst followed by a near zero order delivery of pilocarpine at 20 or 40 μg/h for a period of seven days.

- **Contraception**

Norplant a subdermal implant for long-term delivery of the contraceptive agent levonorgestrel gas recently been approved for marketing by the FDA. The device consists of six silicone membrane capsules each containing about 36 mg of levonorgestel. Cumulatively the six capsules deliver about 70 μg/day at about 800 days.

- **Dental allocation**

Sustained-release fluoride delivery stannous fluoride was incorporated into different dental cements. The device, about 8 mm long and containing 42 mg of fluoride in the core was attached to the buccal surface of the maxillary first molar and designed to release 0.5 mg of fluoride per day for 30 days.

- **Immunization**

The concept here is to provide pulsatile of continuous administration of the antigen over a prolonged time period. For example, immunization efficiency of ethylene-vinyl acetate copolymer pellets containing bovine serum albumin as model antigen.

- **Cancer**

Silicone rod implants similar to those used for delivery of levonorgestrone, testosterone propionate or ethinyl estradiol were used in patients with prostate cancer.

Lupron depot is an implantation system providing one month depot release of leuprolide acetate a synthetic analogue of the gonadotropin releasing hormone (GhRH). The implant consists of biodegradable microspheres prepared from polylactic – glycolic copolymer at 50:50 composition containing 10% leuprolide acetate for the treatment of prostate cancer

- **Narcotic antagonists**

Naltrexone freebase and its hydrochloride or the pamolate acid salt has been prepared in a variety of polymers and dosage forma for prolonged narcotic antagonist activity. Though in vitro delivery of up to 50 day has been achieved by some of the systems in vivo duration of release has been shorter.

1.7 DIFFERENT TYPES OF IMPLANTS

- **Brain implants**[8]

Brain implants, often referred to as neural implants, are technological devices that connect directly to a biological subject's brain- usually placed on the surface of the brain or attached to the brain's cortex. A common purpose of modern brain implants and the focus of much current research is establishing a biomedical prosthesis circumventing areas in the brain that have become dysfunctional after a stroke or other head injuries. This includes sensory

substitution, e.g. in vision. Other brain implants are used in animal experiments simply to record brain activity for scientific reasons. Some brain implants involve creating interfaces between neural systems and computer chips.

- **Microchip implants**[9]

A human microchip implant is an integrated circuit device or RFID (radiofrequency identification) transponder encased in silicate glass and implanted in the body of a human being. A subdermal implant typically contains a unique ID number that can be linked to information contained in an external database, such as personal identification, medical history, medications, allergies, and contact information.

- **Microdermal implant**[10]

Micro dermal implants are a form of body modification which gives the anaesthetic appearance of a transdermal implant, but without the complications of the much more complicated surgery associated with transdermal implants. Microdermal implants can be placed practically anywhere on the surface of the skin on the body.

- **Contraceptive implants**[11]

Norplant is implanted under the skin in the upper arm of a woman, by creating a small incision and inserting the capsules in a fan-like shape. Insertion of Norplant usually takes 15 minutes and the capsules can sometimes be seen under the skin, although usually they looks like small veins.

- **Retinal implants**[12]

A retinal implants is a biomedical implant technology that it meant to partially restore useful vision to people who have lost due to a degenerative eye conditions such as retinitis pigmentosa or macular degeneration. There are two types of retinal implants: epiretinal implant (on the retina) and subretinal implant (behind the retina).

- **Dental implants**[13]

A dental implant is a "root" device, usually made of titanium, used in dentistry to support restorations that resemble a tooth or group of teeth to replace missing teeth. All dental implants placed today are root-form endosseous implants, i.e., they appear similar to an actual tooth root and are placed within the bone. The bone of the jaw accepts and

osseointegrates with the titanium post. Osseointegration refers to the fusion of the implant surface with the surrounding bone. Dental implants will fuse with bone; however they lack the periodontal ligament, so they will feel slightly different than natural teeth during chewing.

Dental implant is available as patches for the bacterial infections in order to prevent the growth of micro-organisms.

DENTAL IMPLANTS

Dental implants can be used to support a number of dental prostheses, including crowns, implant-supported bridges or dentures[14]. They can also be used as anchorage for orthodontic tooth movement. The use of dental implants permits unidirectional tooth movement without reciprocal action.

Dental implants are not susceptible to dental caries and other bacterial infections but they can develop a condition called peri-implantitis. This is an inflammatory condition of the mucosa and/or bone around the implant which may result in bone loss and eventual loss of the implant. The condition is usually, but not always, associated with a chronic infection. Peri-implantitis is more likely to occur in heavy smokers, patients with diabetes, patients with poor oral hygiene and cases where the mucosa around the implant is thin[15].

Dental diseases may affect the teeth or the gums or other tissues and parts of the mouth. Dental diseases can cause much more serious problems than a toothache; they can affect our ability to chew, smile, or speak properly. Their severity may range from a simple apthous ulcer, to a common tooth cavity, or up to a deadly oral cancer. These are among the most common diseases in humans and include dental caries, gingivitis, periodontitis and many more oral conditions[16].

1.9 PERIODONTAL DISEASES[17]

Periodontal diseases are generally divided into two groups

- Gingivitis , which causes lesions (inflammatory abnormalities) that affect the gums.
- Periodontitis , which damages the bone and connective tissue that supports the teeth.

A periodontal disease is caused by bacteria. Even in healthy mouth, the sulcus is teeming with bacteria, but they tend to be harmless varieties. Periodontal diseases usually develops

because of two events in the oral cavity: an increase in bacteria quantity and a change in balance of bacterial types from harmless to disease-causing bacteria.

Periodontitis

Periodontitis occurs when the gum tissues separate from the tooth and sulcus, forming periodontal pockets. Periodontitis is characterized by:

- Gum inflammation, with redness and bleeding
- Deep pockets (greater than 3 mm in depth) that form between the gum and the tooth.
- Loose teeth, caused by loss of connective tissue structures and bone.

Periodontal treatment approaches can basically be categorized as:

- Non-surgical approaches- Scaling and root planning, which may include the use of topical or systemic antibiotics.
- Surgical approaches- Periodontal surgical techniques include flap surgery (periodontal reduction), bone grafts and guided tissue regeneration.
- Restorative procedures- Crown lengthening is an example of a restorative procedure that may be performed for cosmetic reasons or to improve function. For patients who have already lost teeth to advanced periodontitis, dental implants are another options.

Non- Surgical treatment

Scaling and root planning is a deep cleaning to remove bacterial plaque and calculus (tartar). It is the cornerstone of periodontal disease treatment and the first procedure a dentist will use. Scaling involves scraping tartar from the above and below the gum line. Root planning smoothes the root surfaces of the teeth.

At the time of scaling and root planning, the antibiotics are recommended because of the risk of developing antibiotic-resistant infections. Antibiotics for periodontal disease come in various forms. They may be taken as a prescription mouthwash rinse, or placed topically as dissolving gels, threads or microchips into the periodontal pockets.

Nonsurgical periodontal therapy is used to delay repopulation of pathogenic microorganisms by controlling the supragingival bacterial plaque and by disrupting or removing the subgingival gram-negative flora.

The clinical outcome of periodontal treatment is improved if specific microbial pathogens are eradicated from the tissues[18]. Because local scaling and root planning alone

cannot achieve this predictably, mechanical treatment has been combined with the delivery of either topical or systemic antimicrobial therapy[19-21]. Agent used in this situation include amoxicillin, clindamycin, metronidazole, tetracycline and doxycycline. Some antibiotics have been shown to have anti-inflammatory properties that are independent of their antimicrobial activities[22, 23].

In addition to a direct antibacterial effect on ribosomal nits, clindamycin has a number of unique pharmacologic features that enhance its clinical efficacy. Clindamycin is the only proven antibiotic that reduces the adherence of bacteria to the epithelial cell of mucosal surfaces and inhibits the expression of virulence factors.

Clindamycin is known to have a very favourable spectrum of activity against anaerobic infections. Its antimicrobial spectrum also includes gram-positive cocci, gram-positive and gram-negative anaerobes and certain protozoa. Clindamycin has been considered a suitable antimicrobial for the management of periodontal infection. Clindamycin in gel form have been used in the treatment of periodontal diseases[24]. When it was given in gel form its bioavailablity reduces and number of doses increases. Normally clindamycin half-life is 2-3 hrs[25]. In the present study, in order to increase its half-life as well as to reduce the dose, implant has been developed by using the biodegradable polymers.

2. REVIEW OF LITERATURE

2.1 CLINDAMYCIN[24]

Clindamycin is a lincosamide antibiotic which inhibits the growth of the microorganisms. Clindamycin hydrochloride is the hydrated hydrochloride salt of clindamycin, a substance produced by the chlorination of linocomycin. It has a potency equivalent to not less than 800µg of clindamycin ($C_{18}H_{33}ClN_2O_5S$) per mg.

Table 2.1: Characteristics of Clindamycin Hydrochloride

Generic name	Clindamycin Hydrochloride
Synonyms	Anti-clindamycin, Cleocin, Dalacin
Chemical name	L-threo-α-D-galacto-octopryanoside methyl 7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)-carbonyl)amino]-1-thio-(2S-trans) monohydrochloride
Molecular formula	$C_{18}H_{33}ClN_2O_5S.HCl$
Molecular weight	461.45
Appearance	Yellow, amorphous solid

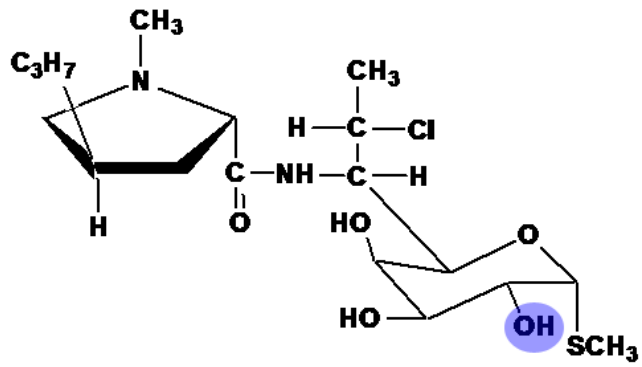


Figure 2.1 Clindamycin Hydrochloride

Mechanism of action

Clindamycin has a bacteriostatic effect. It is a bacterial protein synthesis inhibitor by inhibiting ribosomal translocation[26]. It binds to 50S rRNA of the bacterial ribosome subunit[27]. It also inhibit the binding of amino-acyl transfer RNA or the translocation of messenger RNA Clindamycin contains a basic pyrrolidene ring attached to a sugar group through an amide bond. The replacement of the hydroxyl group in lincomycin to a chloride atom increases the lipophilicity and therefore clindamycin shows a better absorption and penetration into bacterial cells.

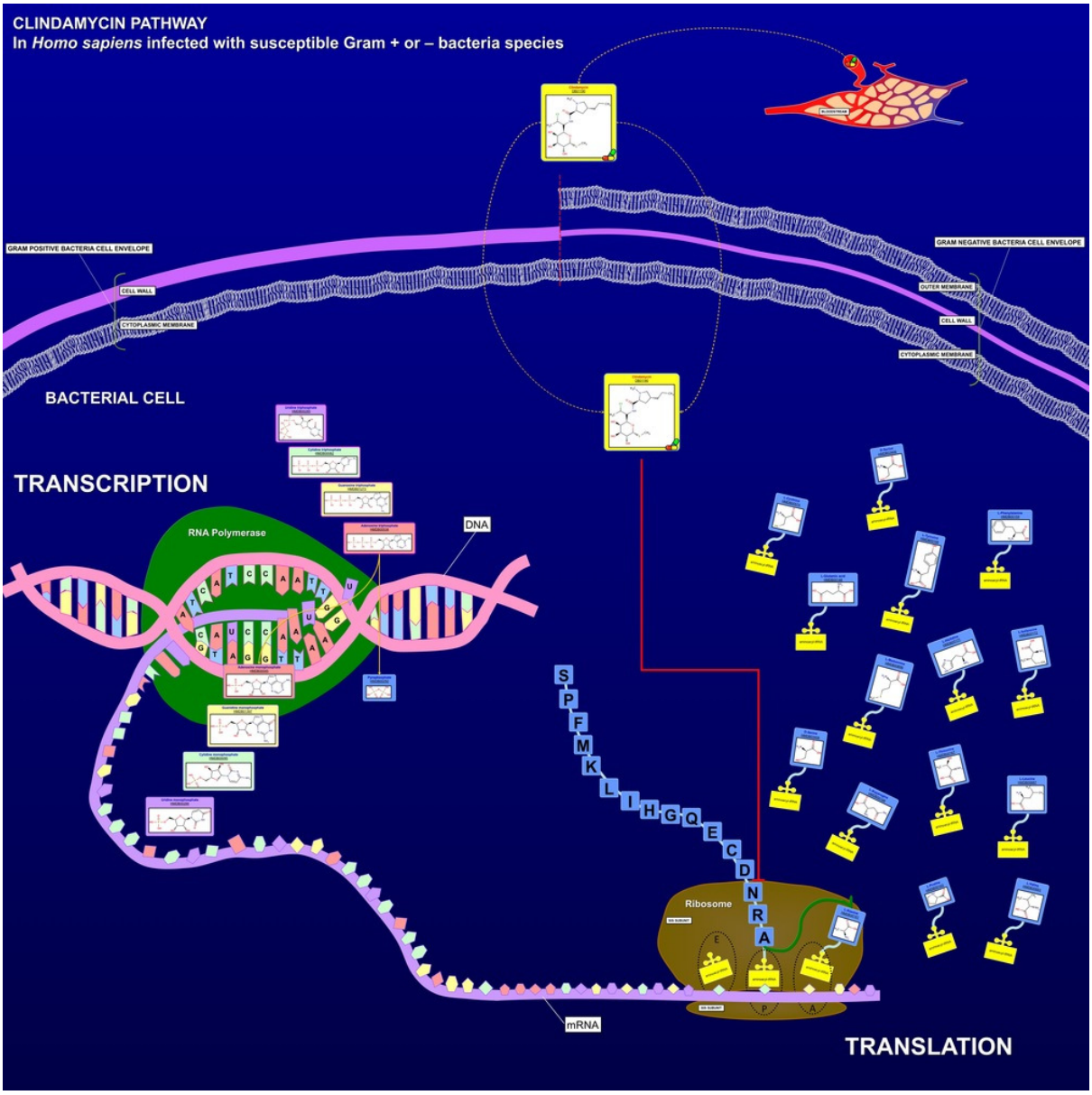


Figure 2.2: Mechanism pathway of Clindamycin hydrochloride[28, 29]

Indication [25]

It is used for the treatment of serious infections caused by susceptible anaerobic bacteria, including *Bacteroides spp*., *Peptostreptococcus*, anaerobic *Streptococci*, *Clostridium spp.,* and microaerophilic *Streptococci*. It may be useful in polymicrobic infections such as intra-abdominal or pelvic infections, osteomyelitis, diabetic foot ulcers, aspiration pneumonia and dental infections. It can also be used to treat MSSA (*Methicillin sensitive Staphylococcus aureus*) and respiratory infections caused by *S. pneumoniae* and *S. pyogenes* in patients who are intolerant to other indicated antibiotics or who are infected with resistant organism. It may be used vaginally to treat vaginosis caused by *Gardnerella vaginosa*. Clindamycin reduces the toxin producing effects of *S. aureus* and *S. pyogenes* and as such, may be particularly useful for treating necrotizing fasciitis.

Table 2.2: Physicochemical and biopharmaceutics parameters of Clindamycin Hydrochloride[25]

Physicochemical parameters	
Storage	Store at controlled room temperature 20ºC to 25ºC (68º to 77ºF)
Solubility	Soluble in water, pyridine, ethanol and DMF (N,N-dimethlyformamide)
Melting point	142ºC
Biopharmaceutical parameters	
Bioavailability	90% (Oral), 4-5%(Topical)
Absorption	Rapidly absorbed after oral administration with peak serum concentrations observed after about 45 minutes. Absorption of an oral dose is virtually complete (90%)
Protein Binding	92-94%
Metabolism	Hepatic
Half life	2-3Hrs
Excretion	Excreted in the urine and in the feces; the remainder is excreted as bioinactive metabolites

Adverse reaction[30]

Gastrointestinal - Abdominal pain, nausea, vomiting, diarrhea, colitis, esophagitis and esophageal ulcer.

Hypersensitivity Reactions - Maculopapular rash and urticaria have been observed during drug therapy. Rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been associated with clindamycin.

Liver - Jaundice and abnormalities in liver function tests have been observed during clindamycin therapy.

Skin and Mucous Membranes - Pruritus, vaginitis and rare instances of exfoliative and vesiculobullous dermatitis have been reported. Rare cases of toxic epidermal necrolysis have been reported during post-marketing surveillance.

Hematopoietic - Transient neutropenia (leukopenia) and eosinophilia have been reported. Reports of agranulocytosis and thrombocytopenia have been made.

Renal - Although no direct relationship of clindamycin to renal damage has been established, renal dysfunction as evidenced by azotemia, oliguria and/or proteinuria has been observed in rare instances.

Musculoskeletal - Rare instances of polyarthritis have been reported.

Nervous System - Dysgeusia has been reported during post-marketing surveillance.

Interactions

Erthyromycin - Antagonistic effect

Kaolin/ Pectin - Decreased GI absorption of clindamycin

Hormonal contraceptives- Decreased contraceptive efficacy

Neuromuscular blockers - Enhanced neuromuscular blockade

Uses

- Acne
- Bacterial vaginosis
- As prophylaxis in bacterial endocarditis
- Other serious anaerobic infection

Dosage form

Capsule	75mg, 150mg, 300mg

Gel, topical 1%	30gm, 60gm or 40ml, 75ml
Granules for oral solution	75mg/5ml (100ml)
I.V	300mg (50ml), 600mg (50ml), 900mg (50ml)
Lotion 1%	60ml
Solution, topical 1%	30ml, 60ml
Vaginal suppository	100mg

Dosage

- **For Severe infection caused by sensitive organisms**

  Adults: 300 to 450mg p.o q 6hrs pr 1.2 to 2.7ml/day i.m or i.v two to four equally divided doses

  Children: 16 to 20mg/kg/day (Hydrochloride) in 3 to 4 equally divided doses or 13 to 25mg/kg/day p.o (Palmitate HCl) in 3 to 4 equally divided doses.

  Neonates younger than 1 month: 15 to 20mg/kg/day i.m or i.v in 3 to 4 equally divided doses.

- **Acute pelvic inflammatory disease**
  Adults: 900mg i.v q 8hrs (given with gentamicin)
- **Acne vulgaris**
  Adults and children older than age 12. Apply a thin film of topical gel, lotion or solution locally to affected area b.i.d.
- **For prevention of endocarditis:**

  Adults: 300 mg orally 1 hour before surgical procedure; then 150 mg 6 hours after initial dose.

  Children: 10 mg/kg (not to exceed adult dose) orally 1 hour before surgical procedure; then 5 mg/kg 6 hours after initial dose.

2.2 POLYMER PROFILE

2.2.1 GELATIN[31]

Gelatin is a generic term for a mixture of purified protein fractions obtained either by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of

animal collagen. The protein fractions consist almost entirely of amino acids joined together by amide linkages to form linear polymers.

Synonyms: Byco; Cryogel; Gelatine, Instagel, Solugel

Molecular weight: 15 000- 2 50 000

Functional categories: Coating agent, film former, gelling agent, suspending agent, tablet binder, viscosity-increasing agent

Pharmaceutical Specifications

Test	Ph Eur 2005
pH	3.8-7.6
Loss of drying	15%
Isoelectric point	6.0-9.5 (Type A), 4.7-5.6 (Type B)

Typical properties

Acidity/alkalinity: For a 1%w/v aqueous solution at 25ºC

pH = 3.8-6.0 (Type A)

pH = 5.0-7.4 (Type B)

Density

1.325 g/cm^3 for type A

1.283 g/cm^3 for type B

Isoelectric point

7-9 for type A

4.7-5.3 for type B

Moisture content: 9-11%

Solubility:

Practically insoluble in acetone, chloroform, ethanol (95%), ether and methanol. Soluble in glycerine, acids and alkalis, although strong acids or alkalis cause precipitation. Gelatine is soluble in hot water, forms a jelly or gel on cooling at 35-40ºC.

Application in pharmaceutical formulation or technology

Gelatin is used as a biodegradable matrix material in an implantable delivery system. Gelatin is also used for the microencapsulation of drugs, preparation of pastes, pastilles, pessaries and suppositories. In addition, it is used as a tablet binder and coating agent and as a viscosity for solutions and semisolids.

2.2.2 SODIUM ALGINATE[32]

Sodium alginate consists of sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid.

Synonyms: Algin, E401, Kelcosol, Keltone, Protanal, Sodium polymannuronate

Functional category: Stabilizing agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity-increasing agent.

Pharmaceutical specifications

Test	USPNF 23
Microbial limits	200/g
Loss of drying	15.0%
Ash	18.0-27.0%
Assay	90.8-106.0%

Typical properties

Acidity/alkalinity: pH~7.2 for a 1%w/v aqueous solution

Solubility:

Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water. Slowly soluble in water and forms a viscous colloidal solution.

Viscosity (dynamic):

Typically, a 1%w/v aqueous solution at 20ºC, will have a viscosity of 20-400 mPas (20-400 cP). Viscosity may vary depending upon concentration, pH, temperature or the presence of metal ions.

Applications in pharmaceutical formulation or technology

Sodium alginate is used in tablet formulations as a binder and disintegrant, it has been used as a diluents in capsule formulations. Sodium alginate has also been used in the preparation of sustained release oral formulations, since it can delay the dissolution of a drug from tablets, capsules and aqueous suspensions.

2.3 **REVIEW OF LITERATURE**

H.A.Khan et al [33], Tamsulosin biodegradable PLGA in-situ implant was formulated and the invitro release study was performed. The effect of drug loading and the effect of excipients on the release pattern were studied. This system is prepared by dissolving a biodegradable polymer DL-poly (lactide-co-glycolide) 70K in biocompatible solvent, dimethyl sulfoxide. Two types of implants were prepared such as implants containing tamsulosin hydrochloride and tamsulosin hydrochloride with biocompatible excipients such as tween 20, tween 60, span 20, span 80, chremophore EL or chremophore RH 40. In vitro dissolution studies were performed in static condition using phosphate buffer (pH 7.4) to observe the release of drugs from these implants for 10 days. Formulation containing only tamsulosin hydrochloride showed that the release rate of drug was 64.51%, 70.64%, 74.08%, 76.12% and 80.05%. It can be concluded that the release rate of drug increases with increasing drug concentrations. The other formulation containing tamsulosin with excipients showed that the release rate was 74.70%, 75.14%, 60.03%, 63.83%, 70.82% and 76.43% against same conc. of drug (8.7% of drug) but different excipients such as tween 20, tween 60, span 20, span 80, chremophore EL and cremophore RH 40 respectively. It can be concluded that excipient lowers the release rate of the drug and may prolong the activity and overall release kinetics.

Heba A Gad et al[34], In Situ implants containing Doxycycline hydrochloride and/or Secnidazole was formulated to treat the periodontitis by direct periodontal intrapocket administration. Biodegradable polymers [poly (lactide) (PLA) and poly (lactide-co-glycolide) (PLGA)], each polymer in two concentrations 25%w/w, 35%w/w were used to formulate the in-situ implants. The rheological behaviour, in vitro drug release and the antimicrobial activity of the prepared implants were evaluated. Increasing the concentration of each polymer increases the viscosity and decreases the percent of the drug released after 24hrs. PLA implants showed a slower drug release rate than PLGA implants in which the implants composed of 25% PLGA showed the fastest drug release.

Ananta Choudhury et al[35], Buccoadhesive film of Ciprofloxacin hydrochloride was developed and in-vitro parameter was studied. Films were formulated using different concentration of hydroxypropyl methyl cellulose and polyvinyl alcohol. The prepared films were subjected to different evaluation like weight determination, thickness, surface pH, folding endurance, swelling index, mucoadhesive time, mucoadhesive strength, drug content, in-vitro drug release study, ex-vivo release study and release kinetic behavior. From the result

it was conclude that all prepared films having desire flexibility and mucoadhesive properties, along with that they shows good in-vitro and ex-vivo drug release performance. Drug release from the films follows desire sustained release phenomenon as needed in buccoadhesive drug delivery.

M.G.Ahmed et al[36], Chitosan strips containing Gatifloxacin (10%, 20% and 30% to the weight of polymer) were prepared by solution casting method using 1% v/v acetic acid in water. The strips containing 30% gatifloxacin were cross-linked by exposing to the vapours of 2% v/v glutaraldehyde in water intended to extend the release. The prepared films were evaluated for their thickness, content uniformity, weight variation, tensile strength, hardness and in-vitro dissolution. Macroscopical features revealed that drug was dissolved in the polymer matrix rather than dispersing. The average weight and thickness of both the crosslinked and uncross-linked strips were uniform. There was a reduction in the tensile strength and increase in hardness when the films were cross-linked. Static dissolution studies showed a burst release initially followed by a progressive fall in the release of the drug and extended upto 19 days once the strip was cross-linked. Release kinetics of gatifloxacin from chitosan strips followed the higuchi's diffusional model and also showed zero order release profile.

Manoj kumar et al[37], Periodontal films containing metronidazole were prepared by solvent casting technique using ethyl cellulose, hydroxyl propyle cellulose and eudragit RL-100 with dibutyl phthalate and polyethylene glycol 400. The films were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, tensile strength and surface pH. Data of in-vitro release from films were fit to different equations and kinetic models to explain release kinetics. Hixon-crowell, Higuchi and Korsmeyer-Peppas models were used to fit the in-vitro release data. Formulation with high concentration of ethyl cellulose released 94.18% of the drug at the end of 120hrs and was more sustained with first order release kinetics. From the result, it was concluded that metronidazole could be incorporated in a slow release device for the treatment of peridontitis.

N.Udupa et al[38], Dental implants of doxycycline hydrochloride and tinidazole were formulated using a biodegradable carrier poly (ε-caprolactone), for the treatment of peridontitis. The in-vitro drug release pattern and stability of these devices were studied. The formulations showed an initial burst release followed by more sustained release of the drugs throughout the period of study (42 days). The stability of the drug were shown a marked improvement by formulating them in polymer matrix.

Varinder kumar et al[39], Mucoadhesive buccal patches containing venlafaxine were prepared using the solvent casting method. Chitosan and pectin were used as bioadhesive polymer at different ratios. The patches were evaluated for their physical characteristics like mass variation, drug content uniformity, folding endurance, surface pH, and in vitro drug release, in vitro buccal permeation study, ex vivo bioadhesion strength and ex vivo mucoadhesion time. Patches exhibited controlled release and releases the entire contents with a period of 10 hrs. Incorporation of PVP K-30 generally enhances the release rate. Swelling index was proportional to the concentration of chitosan. Drug with 1:4 (chitosan:pectin) polymer showed satisfactory bioadhesive strength of 17.53 ± 0.47 g, and ex vivo mucoadhesion time of 10.32hrs. The surface pH of all batches was within ± 0.4 units and thus no mucosal irritation is expected. Patches containing 1:4 of chitosan and pectin had higher bioadhesive strength with sustained drug release as compared to patches with other ratios of polymer. The optimized patch demonstrated good in vitro and ex vivo results.

G.L.Prabushankar et al[40], Levofloxacin dental films for periodontitis were formulated by solvent casting technique using ethyl cellulose and other copolymers in chloroform: dichloromethane (1:1) solvent with dibutyl phthalate and PEG 400. The films were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, tensile strength, surface pH, and in vitro antibacterial activity. In vitro release from films was fit to different equations and kinetic models to reveal release kinetics. The R^2 values are higher for Higuchi's model compared to Hixson Crowell cube root law for all the films and the release from all the films followed diffusion rate. Formulation with eudragit RL-100 released 99.74% of drug at the end of tenth day and was considered as best formulation. . In vitro antibacterial activity was carried out on *S. aureus and E.coli* was found to be effectively higher in 48hrs and then decline at 96hrs.

V.S. Mastiholimath et al[41], Ornidazole dental implants for the treatment of periodontal diseases was prepared by using solvent casting technique using hydroxyl propyl cellulose, hydroxyl methyl cellulose, eudragit RL-100 and ethyl cellulose with dibutyl phthalate . The physicochemical parameters like thickness, weight variation, content uniformity and release characteristic were evaluated. The drug release was initially high on day one to achieve immediate therapeutic level of drug in periodontal pocket followed by marked fall in release by day two with progressive moderate release profile to maintain therapeutic level following anomalous transport mechanism. Formulation with ethyl cellulose released 97.07% of drug at the end of 120hrs and was considered as best formulation. In vitro antibacterial activity was carried out on *Streptococcus mutans* and had an inhibitory effect upto 96hrs.

Rao.K.Purushotham et al[42], Diclofenac sodium biodegradable drug implant for speedy fracture healing was formulated in varied ratios of gelatin and sodium alginate 70:30, 80:20 and 90:10% w/w by heating and congealing method. The implants were evaluated for content uniformity, thickness, weight variation, IR and invitro release studies. The implants gave uniform result for thickness, weight variation and drug content. From the invitro release studies, it was concluded that subdermal implants containing 90:10%w/w gelatin: sodium alginate were found to produce the most satisfactory drug release of 98.41% in 144 hours.

M.G.Ahmed et al[43], Chitosan based Ciprofloxacin and Diclofenac film for peridontitis therapy was prepared by solvent casting method. Some of the drug loaded films were crosslinked with 2% glutaraldehyde for 2 and 4hrs. The films were evaluated for their physicochemical properties including weight variation, thickness, tensile strength, invitro release and antibacterial activity. Mean weight and thickness data showed that the different films were uniform. Tensile strength was maximum for drug-free films and minimum for films containing the highest amount of drug. In vitro drug release data indicate that the films showed an initial burst release followed by sustained release for upto 7 days for uncrosslinked films (87-95%) and 21 days for crosslinked films (70-78%). Films stored at refrigerated conditions exhibited slower degradation rate. The drug loaded films that were crosslinked for 4hrs had inhibitory effect on *S. mutans* for up to 24 days.

Sujatha et al[44], Sparfloxacin dental implants for the treatment of Periodontal diseases was prepared by using solvent casting technique using hydroxyl propyl cellulose, hydroxyl methyl

cellulose, eudragit RL-100 and ethyl cellulose with dibutyl phthalate. The drug release was initially high on day one to achieve immediate therapeutic level of drug in periodontal pocket followed by marked fall in release by day two with progressive moderate release profile to maintain therapeutic level following anamolous transport mechanism. Formulation F4 released 90.24% of drug at the end of 120hr and was considered as best formulation. In vitro antibacterial activity was carried out on *Streptococcus mutans.*

3. AIM AND OBJECTIVE

The goal of drug delivery system is to provide a therapeutic amount of drug to the targeting site in the body to achieve promptly and then maintain the desired drug concentration.

Our system of interest is to develop Clindamycin hydrochloride Implantable drug delivery system. This drug has low half-life of 2-3 hrs, in order to increase its half-life, implants were developed.

Present research work is to design and evaluate the biodegradable implantable drug delivery system of Clindamycin hydrochloride for periodontal diseases.

Thus objective of my work is to formulate and evaluate the implants of Clindamycin hydrochloride in different ratios of drug and polymer, gelatin and sodium alginate and to study the drug release and anti-microbial activity.

4. PLAN OF WORK

- Selection of drug and polymer
- Preformulation study
- Formulation of Clindamycin hydrochloride implant in different drug:polymer ratio using gelatin and sodium alginate.
- Construction of standard graph of pure drug Clindamycin hydrochloride in 0.1N HCl and Phosphate buffer pH 7.4.
- Infra red spectroscopy and differential scanning calorimeter analysis for identifying the interaction between the drug and polymer.
- Physicochemical parameters
  - Weight uniformity test
  - Thickness
  - Drug content uniformity
  - Percentage moisture loss
  - Surface pH
- Invitro drug release studies
- Stability studies
- Antimicrobial activity.

5.MATERIALS AND METHODS

Table 5.1: Materials used in the formulation

Materials	Source
Clindamycin HCl	A to Z Pharmaceuticals Private Ltd, Chennai.
Gelatin	Sd fine – chem. Ltd, Mumbai.
Sodium alginate	Sd fine – chem. Ltd, Mumbai.
PEG 400	Himedia Laboratories Pvt. Ltd, Mumbai.
Potassium dihydrogen phosphate	Spectrum reagents and chemicals Pvt.Ltd, Cochin.
Disodium hydrogen phosphate	Sd fine – chem. Ltd, Mumbai.
Luria Bertani broth	Himedia Laboratories Pvt. Ltd, Mumbai.
Luria Bertani agar	Himedia Laboratories Pvt. Ltd, Mumbai.

Table 5.2: Equipments

S.No	Equipment	Manufacturer
1	IR	Shimadzu, Japan
2	Digital balance	Essae Teraoka Ltd, India
3	UV-Visible	Lab India, Mumbai.
4	Screw gauge	Micrometer, Chennai.
5	Mechanical stirrer	Remi Motor Ltd, Mumbai
6	Differential scanning calorimeter	TA instrument, US.
7	Laminar air flow chamber	Klenzaids, Mumbai.

FORMULATION OF CLINDAMYCIN HCl

The formulae for the preparation of Clindamycin HCl implants were given in the table 6. Gelatin and Sodium alginate were used as a polymer.

Aqueous solution of gelatin and sodium alginate combination were prepared by dissolving them in distilled water by stirring at a water bath at 60ºC. A solution of drug was added to the above aqueous solution. The resultant solution was poured on a glass petridish and allowed to dry at room temperature for 3 days. After drying they were stored at room temperature for further use.

The concentration of polymers (gelatin and sodium alginate) was varied i.e., 500 mg, 750 mg and 1000 mg. In that different concentration of polymers, each had different ratios of gelatin and sodium alginate 70:30, 80:20 and 90:10 as shown in table 5.

Table 5.3: Polymer ratios used in the formulation

Polymer 500mg			
	F1 (70:30)	**F2 (80:20)**	**F3 (90:10)**
Gelatin (mg)	350	400	450
Sodium alginate (mg)	150	100	50
Polymer 750mg			
	F4 (70:30)	**F5 (80:20)**	**F6 (90:10)**
Gelatin (mg)	525	600	675
Sodium alginate (mg)	225	150	75
Polymer 1000mg			
	F7 (70:30)	**F8 (80:20)**	**F9 (90:10)**
Gelatin (mg)	700	800	900
Sodium alginate (mg)	300	200	100

Preparation of implants

Soak gelatin in 15ml of distilled water

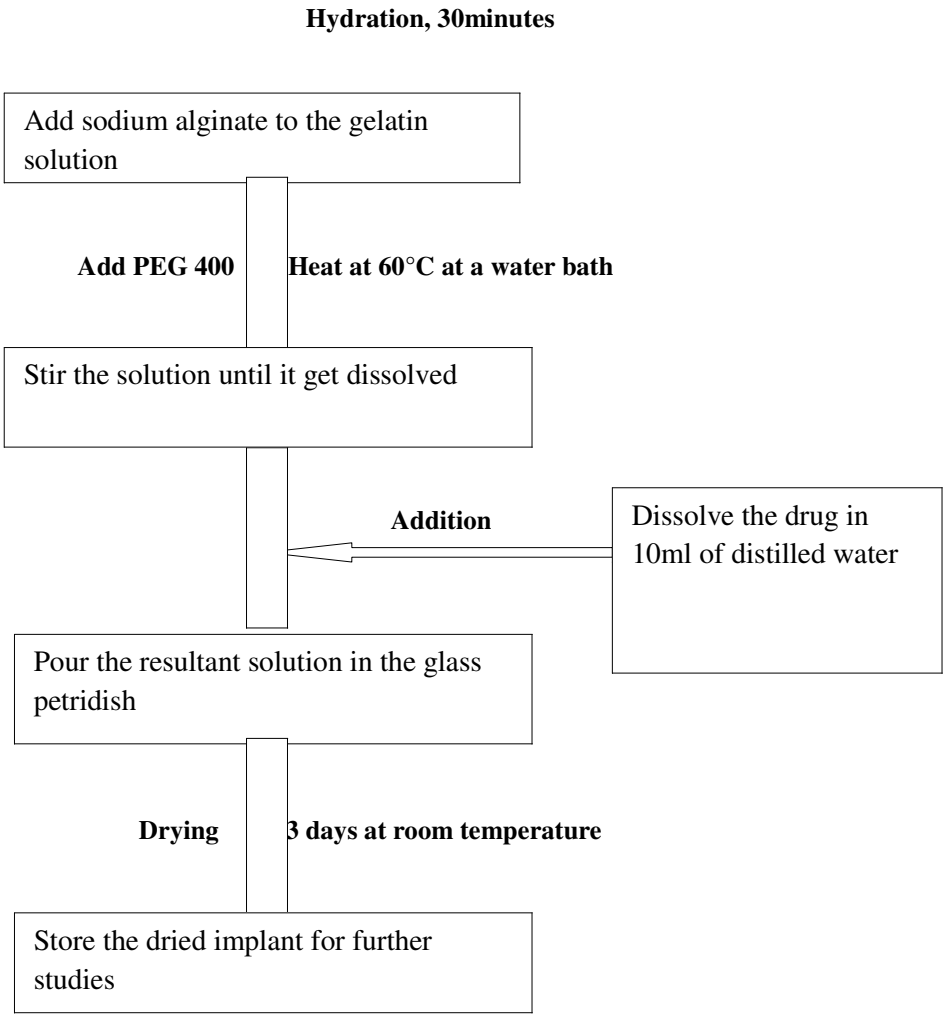


Figure 5.1: Method of preparation of Clindamycin HCl implant

Table 5.4: Formulation chart of Clindamycin hydrochloride implants

Ingredients	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9

Clindamycin hydrochloride (mg)	250	250	250	250	250	250	250	250	250
Gelatin (mg)	350	400	450	525	600	675	700	800	900
Sodium alginate (mg)	150	100	50	225	150	75	300	200	100
PEG 400 (ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Distilled water (ml)	25	25	25	25	25	25	25	25	25

6. EVALUATION

6.1 PRELIMINARY SCREENING

Drug

i)Melting point

Melting point of the sample was determined by using capillary method.

ii)FTIR spectrum

FTIR spectrum of the drug was obtained by preparation of pellets using anhydrous KBr between 4000 cm^{-1} and 500 cm^{-1}.

iii)UV scanning

a) **Scanning of Clindamycin Hydrochloride in 0.1N HCl**
100mg of drug was dissolved in 100ml of 0.1N HCl. 1ml and 5ml of solution was pipette and made upto 100ml with the 0.1N HCl to obtain 10 and 50mg/ml of Clindamycin HCl. Scanning was done in the range 200 to 400nm to obtain λ_{max}.

b) **Scanning of Clindamycin Hydrochloride in pH 7.4 phosphate buffer**
100mg of drug was dissolved in 100ml of pH 7.4 phosphate buffer. 1ml and 5ml of solution was pipette and made upto 100ml with the pH 7.4 phosphate buffer to obtain 10 and 50mg/ml of Clindamycin HCl. Scanning was done in the range 200 to 400nm to obtain λ_{max}.

Polymer

The following polymers were chosen for the formulation of implants.

- HPMC
- MC
- EC
- Gelatin
- Sodium alginate

The trial formulation of implant was done with those polymers and the polymer has been selected. The selected polymer was tested for the compatability studies by using IR and DSC analysis.

6.2 ANALYTICAL METHODS

Construction of standard curve by UV method

Preparation of stock solution

100 mg of Clindamycin hydrochloride was accurately weighed and dissolved in 0.1 N HCl. The volume was made up with the same to produce 100 ml of stock solution having concentration of 1 mg/ml. From this 10 ml was pipette out and made up to 100 ml using 0.1 N HCl having concentration of 100 µg/ml.

Preparation of the working standard

Working standard solutions having concentration of 10 to 60 µg/ml were prepared by appropriately diluting the stock solution with 0.1 N HCl. The absorbance of each working standard was measured at 210 nm in UV spectrophotometer using 0.1 N HCl as a reagent blank.

6.3 DRUG-POLYMER COMPATILIBILITY

6.3.1 IR analysis

Pure drug and polymers were subjected to IR studies. About 2 mg of pure drug/combination of drug-polymer were triturated with KBr (Potassium bromide) to form a pellet. The mixture was placed in the sample holder and was analyzed by infrared to study the interference of polymers with the drug.

6.3.2 DSC analysis

DSC analysis was done to ascertain the compatibility of drug with the excipients.It was performed on a DSC Q10 V 9.0, differential scanning calorimeter with a thermal analyzer. About 2.3 mg of the powered sample was placed in a sealed aluminium pan, before heating under nitrogen flow (20ml/min) at a scanning rate of 10ºC min-1,from 134.98ºC to 148.56ºC. An empty aluminum pan was used as reference.

6.4 PHYSICOCHEMICAL PARAMETERS

The implants were evaluated for

- Weight uniformity test
- Thickness of the implants
- Content uniformity
- Percentage of moisture content
- Surface pH

6.4.1 Weight uniformity test[45, 46]

The weight uniformity test was carried out by weighing 10 patches cut from different places of same formulation and their individual weights were determined by using the digital balance and the average weight was calculated.

Table 6.1: Allowable limit for weight variation

Average weight (mg)	Maximum % difference allowed
130 or less	10
130-324	7.5
More than 324	5

6.4.2 Thickness of film[42]

Film thickness of 10 strips was measured with the help of screw gauge. The strip was placed between the two jaws and the thickness was measured. The mean value was calculated.

6.4.3 Content uniformity

The drug content of the prepared implants was estimated using following method. Implant containing Clindamycin HCl was taken in 100 ml standard volumetric flask. To this pH 7.4 phosphate buffer solution was added and made upto volume. The flask was kept overnight. The solution was filtered and 5 ml of this filtrate was pipetted out into a 100 ml standard volumetric flask and made upto the volume with pH 7.4 phosphate buffer solution and the absorbance was determined at 210 nm.

6.4.4 Percentage moisture loss[47]

The percentage moisture loss was carried out to check integrity of the film at dry conditions. Implant was weighed and kept in a desicator containing anhydrous calcium chloride. After three days, the implants were taken out and reweighed; the % moisture loss was calculated using the formula.

$$Moisture\ loss = (Intial\ weight - Final\ weight / Initial weight) \times 100$$

6.4.5 Surface pH[40]

Implant was allowed to swell for 1 hour on the surface of the agar plate, prepared by dissolving 2% w/v agar in warmed distilled water under stirring and then pouring the solution into the petridish to gelling/solidify at room temperature. The surface pH was measured by means of pH paper placed on the surface of the swollen film. The mean of 3 readings was recorded.

6.5 IN-VITRO DRUG RELEASE STUDIES

The in-vitro release of Clindamycin HCl from the implant was carried out in small test tubes containing 10 ml of pH 7.4 phosphate buffer. The test tubes were sealed with the aluminium foil and kept at 37ºC. The sample was withdrawn and replaced with the fresh pH 7.4 phosphate buffer solution for every 1 hour upto 12th hour. Again the sample was withdrawn at 24th hour. The concentration of drug in the withdrawn solution was measured at 210 nm.

In-vitro drug release kinetic studies[48-51]

The results of in-vitro release profiles obtained for all the formulations were plotted in modes of data treatment as follows,

1. Cumulative % drug released versus time (Zero order kinetic model)
2. Log cumulative % drug remaining versus time (First order kinetic model)
3. Cumulative % drug release versus square root of time (Higuchi plot)
4. Log cumulative % drug release versus log T (Koresmeyer-peppas model)
5. Cube root of drug % remaining versus time (Hixson-Crowell model)

Zero Order Kinetics: Zero order release would be predicted by the following equation.

$$A_t = A_0 - K_0 t$$

Where, A_t = Drug released at time 't', A_0 = Initial drug concentration

K_0 = Zero order rate constant(h^{-1})

First Order kinetics: First order release would be predicted by the following equation

$$\text{Log } C = \text{Log } C_0 - K_t/2.303$$

Where, C = Amount of drug remained at time't', C_0 = Initial amount of drug

K= First order rate constant (h^{-1})

When the data is plotted as log cumulative percent drug remaining versus time it yields a straight line , indicating that the release follows first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with a slope values.

Higuchi Model: In this plot, amount of drug release versus square root of time is plotted. The linearity of regression co-efficient determines whether it is being followed or not.

$$Q= K_H t^{1/2}$$

Where, Q = Amount of drug release at time 't', K_H =Higuchi rate constant

Koresmeyer-peppas model: In order to understand the mode of release of drug from swellable matrices. The data were fitted to the following peppas law equation

$$M_t/M = Kt^n$$

Where, M_t/M = Fraction of drug release at time 't'

K = Constant incorporating the structural and geometrical characteristics of the drug/ polymer system

n = Diffusion exponent related to the mechanism of the release

when the data is plotted as log of drug released versus log time, a straight line is obtained with the slope equal to 'n' and the value of 'K' can be obtained from y-intercept. For fickian release n=0.5 while 0.5<n<1.0 indicates anomalous (Non-fickian) transport and n=1 indicates case II diffusion leading to zero-order release.

Hixson-Crowell model: Data obtained from the invitro release studies were plotted cube root of % remaining versus time.

$$W_0^{1/3} - W_t^{1/3} = Kt$$

Where, W_o = Initial amount of drug release at time't',

W_t = Remaining amount of drug at time't'

K = Constant incorporating the surface-volume relation.

6.6 STABILITY STUDIES OF THE OPTIMIZED FORMULATION

Stability is defined as the ability of particular drug or dosage form in a specific container to remain with its physical, chemical, therapeutic and toxicological specifications. The optimized formulation was selected for the stability studies.

The purpose of stability testing is to provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommended storage conditions, re-test periods and shelf-lives to be determined. In the present study, stability study is carried out for the optimized formulation sealed and packed in aluminium foil at room temperature in dark place and exposure to direct sunlight for one month.

6.7 ANTIMICROBIAL ACTIVITY

6.7.1 Minimum inhibitory concentration[52]

Add 100 µl of Clindamycin solution (100 µg/100 µl) to the first tube. Add 4.0 ml of sterile LB broth to the first tube and add 2.0ml to all other tubes.Transfer 2.0 ml of Luria Bretani broth from the first tube to the second tube. Using a separate pipette, mix the contents of this tube and transfer 2.0 ml to the third tube. Continue dilutions in this manner to tube number 4. Remove 2.0 ml from tube 4 and discard it. The fifth tube, which serves as a control, receives no Clindamycin. Add 20 µl of the bacterial culture to each of the tubes. Incubate all tubes at 37°C for overnight. Finally read the turbidity using spectrophotometry at 600 nm.

6.7.2 Disc-diffusion method[52]

Luria Bertani agar medium were prepared and the test micro-organisms were inoculated by the spread plate method. Filter paper disc were soaked with 2, 4 and 6µg of the Clindamycin implant and placed in the prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24mm from each other, center to center. The agar plates were then incubated at 37°C. After 16 to 18hrs of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameter of the zone of complete inhibition was measured.

6.7.3 Agar well diffusion method[52]

Petriplates containing 20ml Luria Bertani agar medium were seeded with 24hrs culture of bacterial strains. Wells were cut and 2.5, 25, 50, 100 and 200µg of the liquid clindamycin implant were added. The plates were then incubated at 37°C for 24hrs. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

7. RESULT AND DISCUSSION

7.1 PRELIMINARY SCREENING

Drug

i)Melting point

Melting point was found to be 141.8°C. This complies with melting point reported in USP.

ii)FTIR

The obtained IR spectrum of the sample shows all the prominent and primary peaks and confirms Clindamycin HCl.

iii)UV scanning

a) **Scanning of Clindamycin Hydrochloride in 0.1N HCl**
The prepared solution of Clindamycin HCl in 0.1N HCl was scanned in UV spectrophotometer and λ_{max} was found to be 210nm.

b) **Scanning of Clindamycin Hydrochloride in pH 7.4 phosphate buffer**
The prepared solution of Clindamycin HCl in pH 7.4 phosphate buffer was scanned in UV spectrophotometer and λ_{max} was found to be 210nm.

Figure 7.1: λ_{max} of Clindamycin HCl in 0.1N HCl

Figure 7.2: λ_{max} of Clindamycin HCl in pH 7.4 phosphate buffer

Polymer

The implant had formulated with the selected polymers and the results was shown in the following table 7.1

Table 7: Selection of polymer

Trials	Inference
Drug+EC	No implant was formed
Drug+EC+HPMC	No implant was formed
Drug+MC	No implant was formed
Drug+gelatin	No implant was formed
Drug+sodium alginate	No implant was formed
Drug+gelatin+sodium alginate	Implant was formed

From the obtained result, implant was not formed with some polymer due to the solubility nature of the polymers. It was not soluble with the drug during the formulations. Gelatin and sodium alginate was soluble in the drug solution. The trial with gelatin and sodium alginate was done by using different ratios and 70:30, 80:20, 90:10 were selected for the formulations. Further, compatibility study was done by using IR and DSC analysis.

7.2 ANALYTICAL METHOD

7.2.1 Standard curve of Clindamycin HCl in 0.1N HCl

Beer's law was found to be obeyed in the concentration range of 10-60 µg/ml with slope value of 0.005 and correlation coefficient was found to be R^2=0.992. The absorbance values of the working solution in 0.1 N HCl are shown in table no.7.1

Table 7.1: Preparation of standard curve of Clindamycin hydrochloride in 0.1N HCl

Concentration(µg/ml)	Absorbance
10	0.049
20	0.103
30	0.154
40	0.221
50	0.240
60	0.303

Figure 7.3: Standard curve for Clindamycin HCl in 0.1N HCl

Absorbance

7.2.2 Standard curve of Clindamycin HCl in pH 7.4 buffer

Using the same method calibration curve is plotted in pH 7.4 phosphate buffer. Beer's law was found to be obeyed in the concentration range of 10-60 µg/ml with slope value of 0.007 and correlation coefficient was found to be R^2=0.995. The absorbance values of the working solutions in pH 7.4 phosphate buffer are shown in table no.7.2

Table 7.2: Preparation of standard curve of Clindamycin hydrochloride in pH 7.4 buffer

Concentration(µg/ml)	Absorbance
10	0.089
20	0.147
30	0.218
40	0.298
50	0.358
60	0.415

Figure 7.4: Standard curve for Clindamycin HCl in pH 7.4 buffer

Standard graph of Clindamycin HCl in pH 7.4

7.3 DRUG-POLYMER COMPATABILITY

7.3.1 IR analysis

Clindamycin HCl and formulation was subjected for IR spectroscopic analysis, to ascertain whether there is any interaction between the drug and polymers used. The IR spectra obtained are given in figure 7.5 & 7.6 .The characteristic peak of the pure drug were compared with the peaks obtained for formulation and are given in the table 10. From the data, it was observed that similar characteristic peaks at 3379.4, 2959.87, 1451.48, 1683.91, 1255.7, 1081.14 and 714.65cm^{-1} for Clindamycin implants. It appears that there is no chemical interaction between the drug and polymer.

Table 7.3: IR spectral data of pure drug alone and formulation containing Clindamycin HCl

Group	Frequency of pure drug (cm^{-1})	Frequency of formulation (cm^{-1})
N-H Streching	2947.33	2959.87
C-H bending	1447.62	1451.48
N-H stretching	3375.54	3379.4
C=O stretching	1679.09	1683.91
C-Cl stretching	793.73	714.65
C-C stretching	1251.84	1255.7
C-O stretching	1093.67	1081.14

Hence it can be concluded that the drug is in free state and there is no interaction between drug and polymers used.

7.3.2 DSC analysis

In order to investigate the possible interactions between the drug and polymers used, differential scanning calorimetric study as carried out. DSC thermogram of the formulation was compared with the pure drug. The DSC thermograms obtained are reported in Figure 7.7 & 7.8. The pure Clindamycin HCl displayed a sharp endothermic peak at 144.21°C corresponding to the melting

point of the drug and a similar peak was also observed in the formulation. From the DSC thermograms it was observed that the decomposition temperature of the pure drug and the formulation remained the same. Hence it can be concluded that there was no significant interaction between drug and the polymers used.

Figure 7.5: IR Spectrum of pure Clindamycin Hydrochloride

Figure 7.6: IR Spectrum of Clindamycin Hydrochloride with gelatin and sodium alginate

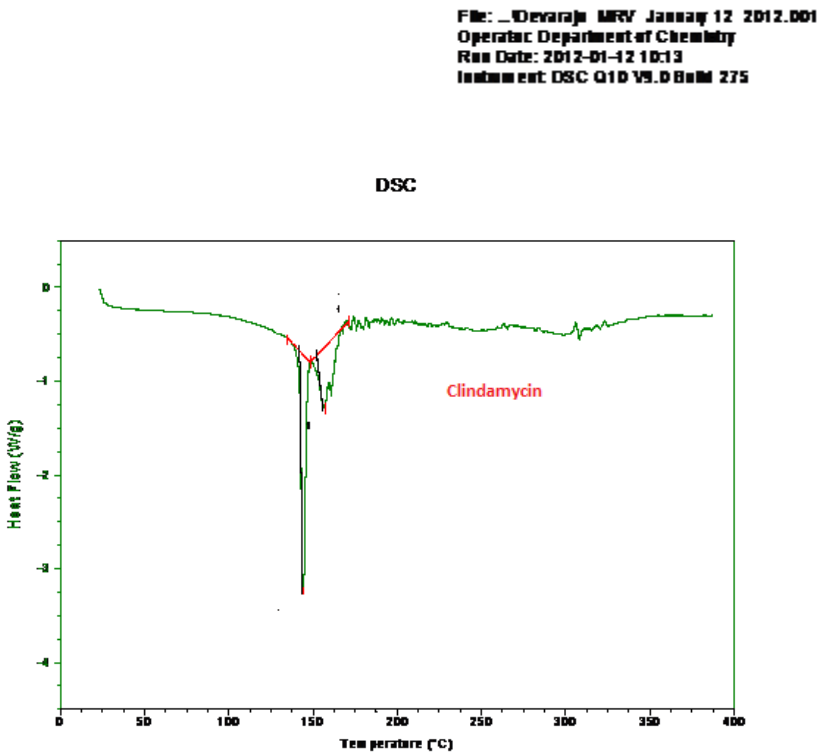


Figure 7.7: Thermogram of pure Clindamycin hydrochloride

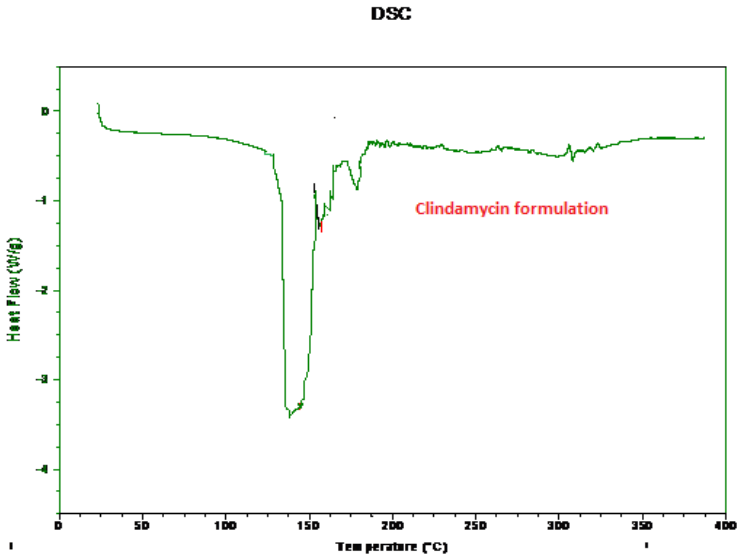


Figure 7.8: Thermogram of Clindamycin HCl with gelatin and sodium alginate

7.4 PHYSICOCHEMICAL PARAMETERS

7.4.1 Weight uniformity test

Table 7.4: Weight uniformity of the Clindamycin HCl implant

Formulation code	Weight of implants(mg)
F1	0.017±0.001
F2	0.020±0.002
F3	0.021±0.002
F4	0.025±0.002
F5	0.027±0.002
F6	0.026±0.002
F7	0.033±0.003
F8	0.029±0.003
F9	0.032±0.003

n=10

Drug loaded films were tested for uniformity of weight. The weight was found to be uniform in the prepared batches.

7.4.2 Thickness of the implants

Table 7.5: Thickness of the Clindamycin HCl implant

Formulation code	Thickness (mm)
F1	0.114±0.0156
F2	0.1±0.01
F3	0.131±0.0074

F4	0.142±0.0104
F5	0.134±0.0112
F6	0.133±0.011
F7	0.169±0.015
F8	0.16±0.0132
F9	0.159±0.0132

n=10

Drug loaded implants were tested for thickness. From the result it was inferred that F1 has least thickness where as the F7 has a highest thickness since it contain highest concentration of polymer in the ratio 70:30, 700mg and 300mg of gelatin and sodium alginate respectively.

7.4.3 Content uniformity

Table 7.6: Content uniformity of the Clindamycin HCl

Formulation code	Trial I (mg)	Trial II (mg)	Trial III (mg)	Average (mg)
F1	10.53	11.56	11.33	11.14±0.40
F2	11.50	11.53	11.30	11.44±0.09
F3	11.50	11.60	11.43	11.51±0.06
F4	11.01	11.46	11.21	11.22±0.15
F5	11.37	10.07	11.05	10.83±0.50
F6	9.93	11.50	11.40	10.94±0.67
F7	11.54	11.51	11.44	11.49±0.04
F8	11.43	11.45	11.53	11.47±0.12
F9	11.48	11.50	11.43	11.47±0.11

The test for content uniformity was carried out to ascertain that the drug is uniformly distributed into formulation.

7.4.4 Percentage moisture loss

Table 7.7: % Moisture loss of the Clindamycin HCl implants

Formulation code	Trial I	Trial II	Average
F1	28%	37.5%	32.75%
F2	50%	42.85%	46.42%
F3	25%	31.80%	28.4%
F4	26.47%	33.33%	29.9%
F5	25.71%	16.13%	20.92%
F6	16.6%	21.62%	19.11%
F7	20%	17.64%	18.82%
F8	19.35%	22.85%	21.1%
F9	21.73%	16.66%	19.19%

From the result it was inferred that F2 has highest moisture loss whereas F7 has lowest moisture loss. If gelatin concentration increases moisture loss decreases, since gelatin has low moisture content.

7.4.5 Surface pH

Table 7.8: Surface pH of the Clindamycin HCl implant

Formulation code	Trial I	Trial II	Trial III	Average
F1	6	6	6	6
F2	6	6	6	6
F3	6	6	6	6
F4	7	6	7	6.6
F5	7	6	6	6.3
F6	7	7	6	6.6
F7	7	6	7	6.6
F8	6	7	7	6.6
F9	7	7	6	6.6

The surface pH of all formulations was within the range of 6-7 which is close to the neutral pH and hence no irritation was expected.

7.4 INVITRO DRUG RELEASE STUDIES

The invitro result of all formulations was carried out in Phosphate buffer pH 7.4. The results are shown in the table 7.9

Table 7.9: In-vitro release data of Clindamycin HCl implants of different formulations

Time in hrs	Formulation code								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1	22.84	23.40	23.96	20.46	22.68	23.20	20.12	21.38	22.06
2	26.01	27.13	28.61	24.41	27.89	30.20	23.01	24.20	24.79
3	44.20	44.76	48.01	26.29	29.41	33.58	32.62	37.61	38.24
4	49.17	54.02	56.27	31.34	34.22	37.63	41.86	43.81	45.43
5	55.11	59.45	63.48	37.85	39.82	41.00	49.43	53.62	55.75
6	59.63	63.52	66.95	43.04	47.61	51.72	54.18	59.56	59.76
7	71.50	75.35	79.67	50.82	54.28	57.47	59.74	62.24	63.69
8	79.87	80.12	81.88	57.63	62.08	63.46	61.64	67.37	68.52
9	85.29	87.45	91.52	62.68	65.85	69.87	68.47	74.97	76.91
10	96.21	97.71	98.23	73.67	76.43	78.57	72.68	78.79	80.20
11	-	-	-	79.53	81.44	82.72	75.53	81.50	83.91
12	-	-	-	91.14	92.98	94.38	79.78	82.62	85.13
24	-	-	-	94.71	96.41	97.27	84.90	87.73	89.77

%drug release

Figure 7.9: In-vitro drug release of the formulation F1, F2 and F3

Figure 7.10: In-vitro drug release of the formulation F4, F5 and F6

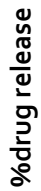


Figure 7.11: In-vitro drug release of the formulation F7, F8 and F9

From the obtained results, the F1, F2, F3 formulations release the drug 96.21, 97.71, 98.23 respectively at 10^{th} hour due to the presence of low polymer concentration.

In the F4, F5, F6 formulation the release of the drug was 94.71, 96.41, 97.27 respectively at 24^{th} hour because the polymer concentration is increased when compared with the F1-F3 formulations.

The polymer concentration again increased to sustain the drug release in the formulations F7, F8, F9 and its release rate was found to be 84.90, 87.73, 89.77 respectively. By increasing the polymer concentration , the drug release can be sustained.

In-vitro drug release kinetics

7.5.1 Formulation No.1

Table 7.10: Kinetics data of invitro drug release for F1

Time in hrs	log T	Square root of T	Cumulative %drug release	Log cumulative %drug release	%drug remaining	Log %drug remaining	Cube root of drug % remaining
0	0	0	0	0	0	0	0
1	0	1	22.84	1.358	77.16	1.887	4.327
2	0.3010	1.414	26.01	1.415	73.99	1.867	4.198
3	0.4771	1.732	44.20	1.645	55.80	1.746	3.821
4	0.6020	2	49.17	1.691	50.83	1.706	3.704
5	0.6989	2.236	55.11	1.741	44.89	1.652	3.553
6	0.7781	2.449	59.63	1.775	40.37	1.606	3.430
7	0.8450	2.645	71.50	1.854	28.50	1.454	3.054
8	0.9030	2.828	79.87	1.902	20.13	1.303	20.720
9	0.9542	3	85.29	1.930	14.71	1.167	2.450
10	1	3.162	96.21	1.983	3.79	0.578	1.559
11	1.0413	3.316	-	-	-	-	-
12	1.0791	3.464	-	-	-	-	-
24	1.3802	4.898	-	-	-	-	-

7.5.2 Formulation No: 2

Table 7.11: Kinetics data of invitro drug release for the F2

Time	log T	Square	Cumulativ	Log	%drug	Log	Cube

in hrs		**root of T**	**e %drug release**	**cumulative %drug release**	**remaining**	**%drug remaining**	**root of drug % remaining**
0	0	0	0	0	0	0	0
1	0	1	23.40	1.369	76.60	1.884	4.246
2	0.3010	1.414	27.13	1.433	72.87	1.862	4.176
3	0.4771	1.732	44.76	1.650	55.24	1.742	3.808
4	0.6020	2	54.02	1.732	45.98	1.662	3.582
5	0.6989	2.236	59.45	1.774	40.55	1.607	3.435
6	0.7781	2.449	63.52	1.802	36.48	1.562	3.316
7	0.8450	2.645	75.35	1.877	24.65	1.391	2.910
8	0.9030	2.828	80.12	1.903	19.88	1.298	2.708
9	0.9542	3	87.45	1.941	12.55	1.098	2.323
10	1	3.162	97.71	1.989	2.29	0.359	1.318
11	1.0413	3.316	-	-	-	-	-
12	1.0791	3.464	-	-	-	-	-
24	1.3802	4.898	-	-	-	-	-

7.5.3 Formulation No: 3

Table 7.12: Kinetics data of invitro drug release for the F3

Time in hrs	log T	Square root of T	Cumulative %drug release	Log cumulative %drug release	%drug remaining	Log %drug remaining	Cube root of drug % remaining
0	0	0	0	0	0	0	0
1	0	1	23.96	1.379	76.04	1.881	4.236
2	0.3010	1.414	28.61	1.456	71.39	1.853	4.148
3	0.4771	1.732	48.01	1.681	51.99	1.715	3.732
4	0.6020	2	56.27	1.750	43.73	1.640	3.523
5	0.6989	2.236	63.48	1.802	36.52	1.562	3.317
6	0.7781	2.449	66.95	1.825	33.05	1.519	3.209
7	0.8450	2.645	79.67	1.901	20.33	1.308	2.729
8	0.9030	2.828	81.88	1.913	18.12	1.258	2.626
9	0.9542	3	91.52	1.961	8.48	0.928	2.039
10	1	3.162	98.23	1.992	1.77	0.247	1.209
11	1.0413	3.316	-	-	-	-	-
12	1.0791	3.464	-	-	-	-	-
24	1.3802	4.898	-	-	-	-	-

7.5.4 Formulation No: 4

Table 7.13: Kinetics data of invitro drug release for the F4

Time in hrs	log T	Square root of T	Cumulative %drug release	Log cumulative %drug release	%drug remaining	Log %drug remaining	Cube root of drug % remaining
0	0	0	0	0	0	0	0
1	0	1	20.46	1.310	79.54	1.900	4.300
2	0.3010	1.414	24.41	1.387	75.59	1.878	4.228
3	0.4771	1.732	26.29	1.419	73.71	1.867	4.192
4	0.6020	2	31.34	1.496	68.66	1.836	4.094
5	0.6989	2.236	37.85	1.578	62.15	1.793	3.961
6	0.7781	2.449	43.04	1.633	56.96	1.755	3.847
7	0.8450	2.645	50.82	1.706	49.18	1.691	3.663
8	0.9030	2.828	57.63	1.760	42.37	1.627	3.486
9	0.9542	3	62.68	1.797	37.32	1.571	3.341
10	1	3.162	73.67	1.867	26.33	1.420	2.974
11	1.041	3.316	79.53	1.900	20.47	1.311	2.735

	3						
12	1.0791	3.464	91.14	1.959	8.86	0.947	2.069
24	1.3802	4.898	94.71	1.976	5.29	0.723	1.742

7.4.5 Formulation No: 5

Table 7.14: Kinetics data of the invitro drug release for F5

Time in hrs	**log T**	**Square root of T**	**Cumulative %drug release**	**Log cumulative %drug release**	**%drug remaining**	**Log %drug remaining**	**Cube root of drug % remaining**
0	0	0	0	0	0	0	0
1	0	1	22.68	1.355	77.32	1.888	4.260
2	0.3010	1.414	27.89	1.445	72.11	1.857	4.162
3	0.4771	1.732	29.41	1.468	70.59	1.848	4.132
4	0.6020	2	34.22	1.534	65.78	1.818	4.036
5	0.6989	2.236	39.82	1.600	60.18	1.779	3.918
6	0.7781	2.449	47.61	1.677	52.39	1.719	3.741

7	0.8450	2.645	54.28	1.734	45.72	1.660	3.575
8	0.9030	2.828	6208	1.792	37.92	1.578	3.359
9	0.9542	3	65.85	1.818	34.15	1.533	3.244
10	1	3.162	76.43	1.883	23.57	1.372	2.867
11	1.0413	3.316	81.44	1.910	18.56	1.268	2.647
12	1.0791	3.464	92.98	1.968	7.02	0.846	1.914
24	1.3802	4.898	96.41	1.984	3.59	0.555	1.531

7.4.6 Formulation No: 6

Table 7.15: Kinetics data of the invitro drug release for the F6

Time in hrs	log T	Square root of T	Cumulative %drug release	Log cumulative %drug release	%drug remaining	Log %drug remaining	Cube root of drug % remaining
0	0	0	0	0	0	0	0
1	0	1	23.20	1.365	76.80	1.885	4.250
2	0.3010	1.414	30.20	1.480	69.80	1.843	4.117
3	0.477	1.732	33.58	1.526	66.42	1.822	4.049

	1						
4	0.6020	2	37.63	1.575	62.37	1.794	3.965
5	0.6989	2.236	41.00	1.612	59.00	1.770	3.892
6	0.7781	2.449	51.72	1.713	48.28	1.683	3.641
7	0.8450	2.645	57.47	1.759	42.53	1.628	3.490
8	0.9030	2.828	63.46	1.802	36.54	1.562	3.318
9	0.9542	3	69.87	1.844	30.13	1.478	3.111
10	1	3.162	78.57	1.895	21.43	1.331	2.777
11	1.0413	3.316	82.72	1.917	17.28	1.237	2.585
12	1.0791	3.464	94.38	1.974	5.62	0.749	1.777
24	1.3802	4.898	97.27	1.987	2.73	0.430	1.397

7.5.7 Formulation No: 7

Table 7.16: Kinetics data of the invitro drug release for F7

Time in	log T	Square root of	Cumulative %drug	Log cumulativ	%drug remainin	Log %drug	Cube root of

hrs		T	release	e %drug release	g	remainin g	drug % remainin g
0	0	0	0	0	0	0	0
1	0	1	20.12	1.303	79.88	1.902	4.306
2	0.301 0	1.414	23.01	1.361	76.99	1.886	4.254
3	0.477 1	1.732	32.62	1.513	67.38	1.828	4.069
4	0.602 0	2	41.86	1.621	58.14	1.764	3.873
5	0.698 9	2.236	49.43	1.693	50.57	1.703	3.697
6	0.778 1	2.449	54.18	1.733	45.82	1.661	3.578
7	0.845 0	2.645	59.74	1.776	40.26	1.604	3.427
8	0.903 0	2.828	61.64	1.789	38.36	1.583	3.372
9	0.954 2	3	68.47	1.835	31.53	1.498	3.159
10	1	3.162	72.68	1.861	27.32	1.436	3.011
11	1.041 3	3.316	75.53	1.878	24.47	1.388	2.903
12	1.079 1	3.464	79.78	1.901	20.22	1.305	2.724
24	1.380 2	4.898	84.90	1.928	15.10	1.178	2.471

Cumulative %drug release

Figure 7.12: Zero-order release kinetics for the formulation F7

Figure 7.13: First-order release kinetics for the formulation F7

Figure 7.14: Higuchi release kinetics for the formulation F7

log cumulative %drug release

Figure 7.15: Koresmeyer-peppas release kinetics for the formulation F7

Figure 7.16: Hixson-Crowell release kinetics for the formulation F7

7.5.8 Formulation No: 8

Table 7.17: Kinetics data of the invitro drug release for F8

Time in hrs	log T	Square root of T	Cumulative %drug release	Log cumulative %drug release	%drug remaining	Log %drug remaining	Cube root of drug % remaining
0	0	0	0	0	0	0	0
1	0	1	21.38	1.330	78.62	1.895	4.283
2	0.3010	1.414	24.20	1.383	75.80	1.876	4.232
3	0.4771	1.732	37.61	1.575	62.39	1.795	3.966
4	0.6020	2	43.81	1.641	56.19	1.749	3.830
5	0.6989	2.236	53.62	1.729	46.38	1.666	3.592
6	0.7781	2.449	59.56	1.774	40.44	1.606	3.432
7	0.8450	2.645	62.24	1.794	37.76	1.577	3.354
8	0.9030	2.828	67.37	1.828	32.63	1.513	3.195
9	0.9542	3	74.97	1.874	25.03	1.398	2.925
10	1	3.162	78.79	1.896	21.21	1.326	2.768
11	1.0413	3.316	81.50	1.911	18.50	1.267	2.644
12	1.079	3.464	82.62	1.917	17.38	1.240	2.590

	1						
24	1.3802	4.898	87.73	1.943	12.27	1.088	2.306

7.5.9 Formulation No: 9

Table 7.18: Kinetics data of the invitro drug release for F9

Time in hrs	log T	Square root of T	Cumulative %drug release	Log cumulative %drug release	%drug remaining	Log %drug remaining	Cube root of drug % remaining
0	0	0	0	0	0	0	0
1	0	1	22.06	1.343	77.94	1.891	4.271
2	0.3010	1.414	24.79	1.394	75.21	1.876	4.221
3	0.4771	1.732	38.24	1.582	61.76	1.790	3.952
4	0.6020	2	45.43	1.657	54.57	1.736	3.793
5	0.6989	2.236	55.75	1.746	44.25	1.645	3.537
6	0.7781	2.449	59.76	1.776	40.24	1.604	3.426
7	0.8450	2.645	63.69	1.804	36.31	1.560	3.311

8	0.9030	2.828	68.52	1.835	31.48	1.498	3.157
9	0.9542	3	76.91	1.885	23.09	1.363	2.847
10	1	3.162	80.20	1.904	19.80	1.296	2.705
11	1.0413	3.316	83.91	1.923	16.09	1.266	2.524
12	1.0791	3.464	85.13	1.930	14.87	1.172	2.459
24	1.3802	4.898	89.77	1.953	10.23	1.009	2.170

From the various mathematical models, the in-vitro kinetics studies were performed for all the formulations. The 'n' value was in the range 0.87-0.93. So that the prepared implant undergo diffusion mechanism because it follows non-fickian or anamolous transport.

7.6 COMPARATIVE KINETICS OF ALL THE FORMULATIONS

The comparative kinetics value for zero-order, first-order, higuchi, koresmeyer-peppas and Hixson-crowell are shown in the following tables and figures.

Table 7.19: Comparative kinetics for Zero-order drug release kinetics

Formulation code	Zero-Order Drug Release	
	R^2	K_0
F1	0.973	10.09
F2	0.968	11.42
F3	0.959	12.81
F4	0.812	18.95
F5	0.801	21.81
F6	0.789	24.03

F7	0.725	26.88
F8	0.696	28.75
F9	0.696	29.46

$$A_t = A_0 - K_0 t$$

Cumulative %drug release

Figure 7.17: Comparative kinetics of Zero-order for the formulation F1, F2 and F3

Figure 7.18: Comparative kinetics of Zero-order for the formulation F4, F5 and F6

Figure 7.19: Comparative kinetics of Zero-order for the formulation F7, F8 and F9

Table 7.20: Comparative kinetics for First-order kinetics

Formulation code	First-Order Drug Release	
	R^2	K_1
F1	0.014	1.468
F2	0.030	1.482
F3	0.057	1.495
F4	0.098	1.656
F5	0.142	1.662

F6	0.178	1.663
F7	0.005	1.534
F8	0.022	1.517
F9	0.038	1.519

$$\text{Log } C = \text{Log } C_0 - K_t/2.303$$

Log cumulative% drug remaining

Figure 7.20: Comparative kinetics of First-order for the formulation F1, F2 and F3

Figure 7.21: Comparative kinetics of First-order for the formulation F4, F5 and F6

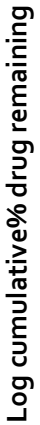


Figure 7.22: Comparative kinetics of First-order for the formulation F7, F8 and F9

Table 7.21: Comparative kinetics for Higuchi release kinetics

Formulation code	Higuchi Drug Release	
	R^2	K_H
F1	0.965	0.048
F2	0.974	0.070
F3	0.979	0.093
F4	0.915	0.063
F5	0.923	0.071
F6	0.929	0.076
F7	0.932	0.026
F8	0.917	0.035
F9	0.917	0.041

$$Q=K_H t^{1/2}$$

Figure 7.23: Comparative kinetics of Higuchi for the formulation F1, F2 and F3

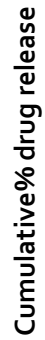


Figure 7.24: Comparative kinetics of Higuchi for the formulation F4, F5 and F6

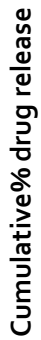


Figure 7.25: Comparative kinetics of Higuchi for the formulation F7, F8 and F9

Table 7.22: Comparative kinetics for Koresmeyer-peppas drug release kinetics

Formulation code	Koresmeyer-peppas Drug Release	
	R^2	K_{kp}
F1	0.648	0.829
F2	0.644	0.841
F3	0.640	0.855
F4	0.668	0.836
F5	0.641	0.876
F6	0.519	0.991
F7	0.636	0.883

F8	0.625	0.908
F9	0.622	0.917

$$M_t/M = Kt^n$$

Log cumulative %drug release

Figure 7.26: Comparative kinetics of Koresmeyer-peppas for the formulation F1, F2 and F3

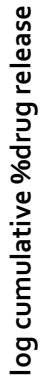


Figure 7.27: Comparative kinetics of Koresmeyer-peppas for the formulation F4, F5 and F6

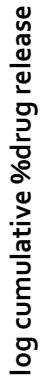


Figure 7.28: Comparative kinetics of Koresmeyer-peppas for the formulation F7, F8 and F9

Table 7.23: Comparative kinetics for Hixson-Crowell drug release kinetics

Formulation code	Hixson-Crowell Drug Release	
	R^2	K_{HC}
F1	0.015	0.048
F2	0.033	0.070
F3	0.056	0.093
F4	0.100	0.063
F5	0.125	0.071
F6	0.143	0.076
F7	0.022	0.026
F8	0.038	0.035

F9	0.054	0.041

$$W_0^{1/3} - W_t^{1/3} = Kt$$

Figure 7.29: Comparative kinetics of Hixson-Crowell for the formulation F1, F2 and F3

Figure 7.30: Comparative kinetics of Hixson-Crowell for the formulation F4, F5 and F6

Figure 7.31: Comparative kinetics of Hixson-Crowell for the formulation F7, F8 and F9

7.6 STABILITY STUDIES

Stability studies of the optimized formulations of Clindamycin implants was carried out to determine the effect of formulation additives on the stability of the drug and also to determine the physical stability of the formulations. The stability studies were carried out at room temperature 29ºC, dark place and direct sunlight.

Stability studies showed after one month the significant changes in the physicochemical parameters and drug release was shown in the table 31.

Table 7.24: Stability studies data of the optimized formulation of Clindamycin implants

S.No	Parameter	Stability studies					
		0th day			30th day		
		Room temp. 29ºC	Dark place	Direct sunlight	Room temp. 29ºC	Dark place	Direct sunlight
1	Weight uniformity	0.032	0.028	0.026	0.030	0.025	0.020
2	Thickness	0.165	0.166	0.152	0.162	0.164	0.150
3	% Moisture loss	16.6%	19.01%	18.21%	16.05 %	18.89 %	17.65%
4	Surface pH	7	6	7	7	6	7
5	Drug content	11.62	11.48	11.29	11.61	11.45	11.05
6	Drug release at 24th hour	85.96%	84.97%	85.69%	86.23 %	85.60 %	90.25%

Stability studies were conducted on implants at room temperature, dark place and exposure to direct sunlight for one month. The observed parameters reduced markedly after exposing to direct sunlight and no significant change at room temperature, dark place. The stability of the drug was improved by formulating them in polymer matrix.

7.7 ANTIMICROBIAL ACTIVITY

7.7.1 Minimum inhibitory concentration

The minimum inhibitory concentration was determined by using different concentration of the Clindamycin implant in various micro-organisms.

Table 7.25: Minimum inhibitory concentration of Clindamycin HCl

Organism name	Control	12.5 µg	25 µg	50 µg	100µg
Klebsiella	0.132	0.113	0.11	0.108	0.084
Salmo	0.15	0.114	0.092	0.087	0.069
SPA	0.143	0.121	0.108	0.097	0.081
SPB	0.138	0.098	0.092	0.085	0.061
Ent.bact	0.109	0.088	0.082	0.071	0.009
Pro mira	0.175	0.114	0.136	0.103	0.093
Staph	0.077	0.008	0.006	0.005	0.004

Kleb – *Klebsiella pneumonia*; Salmo- *Salmonella*; SPA- *Salmonella typhi* A; SPB- *Salmonella typhi* B; Ent.bact-Enterobacter aerogens; Pro mira- *Proteus mirabilis*; Staph- *Staphylococcus aureus*

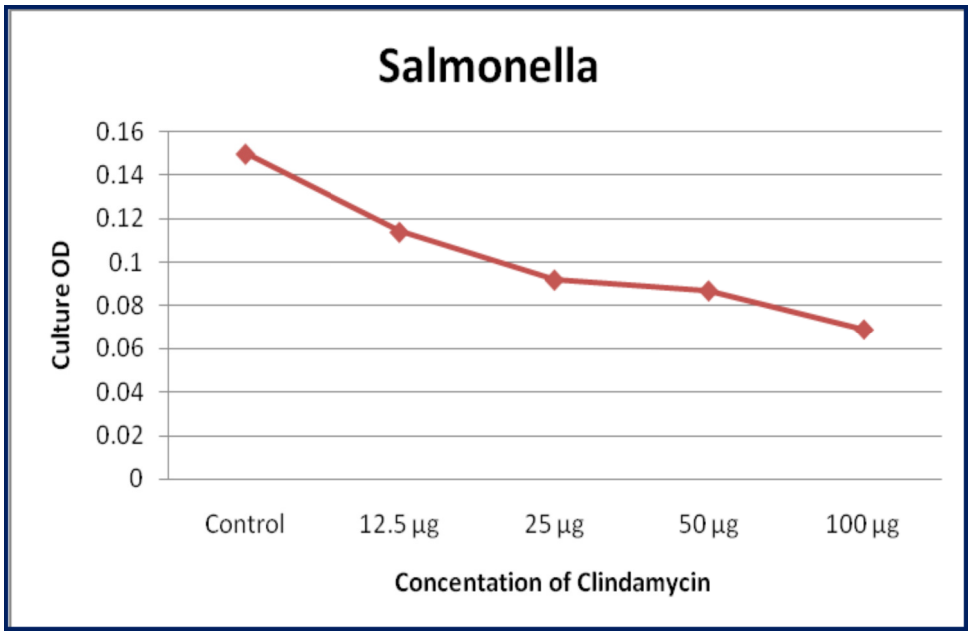


Figure 7.32: MIC of Clindamycin implant in *Salmonella*

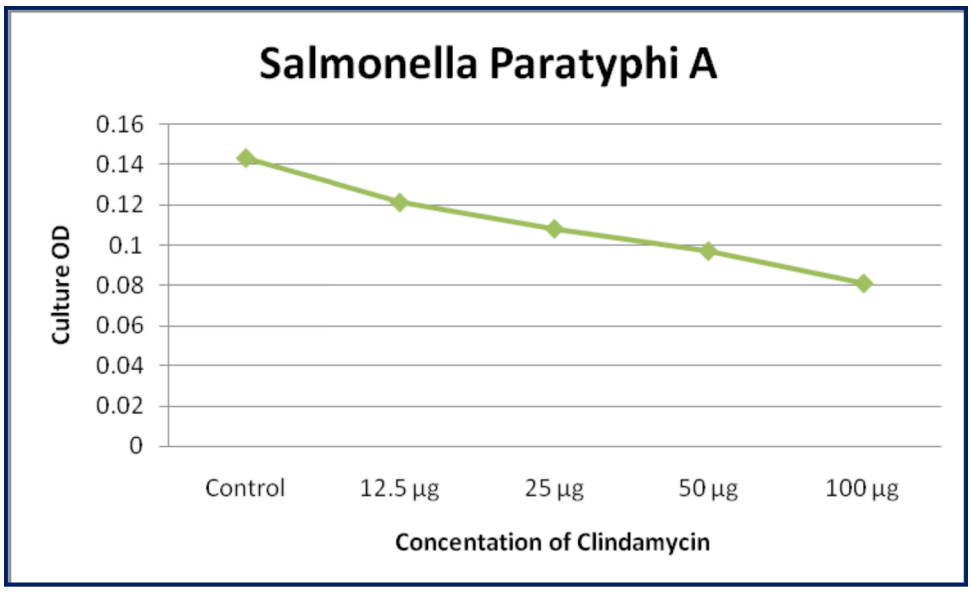


Figure 7.33: MIC of Clindamycin implant in *Salmonella paratyphi A*

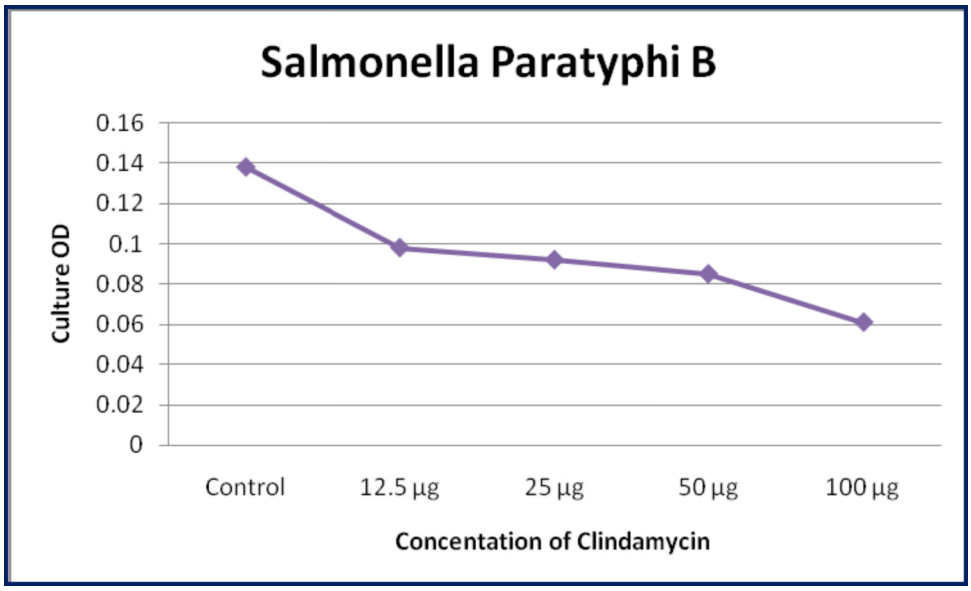


Figure 7.34: MIC of Clindamycin implant in *Salmonella paratyphi B*

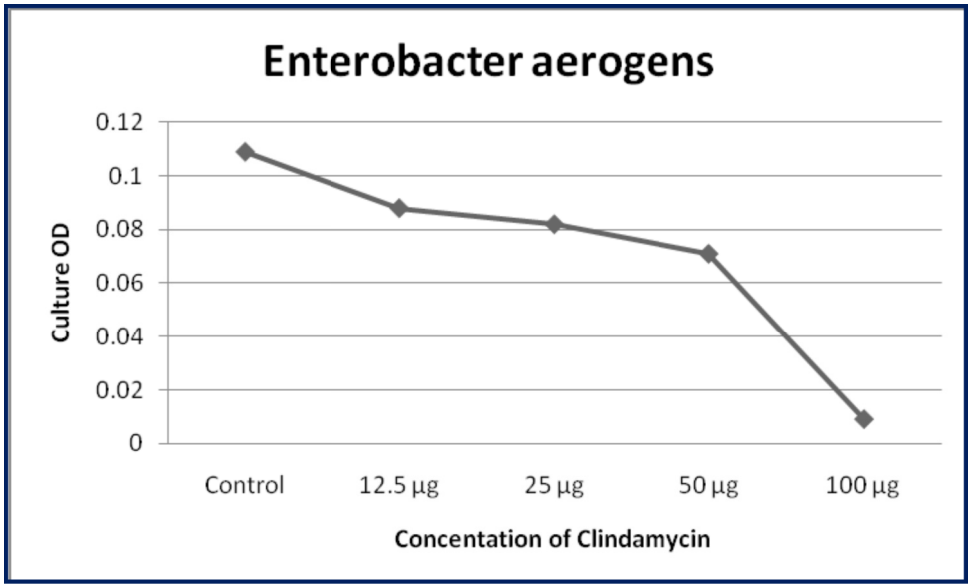


Figure 7.35: MIC of Clindamycin implant in *Enterobacter aerogens*

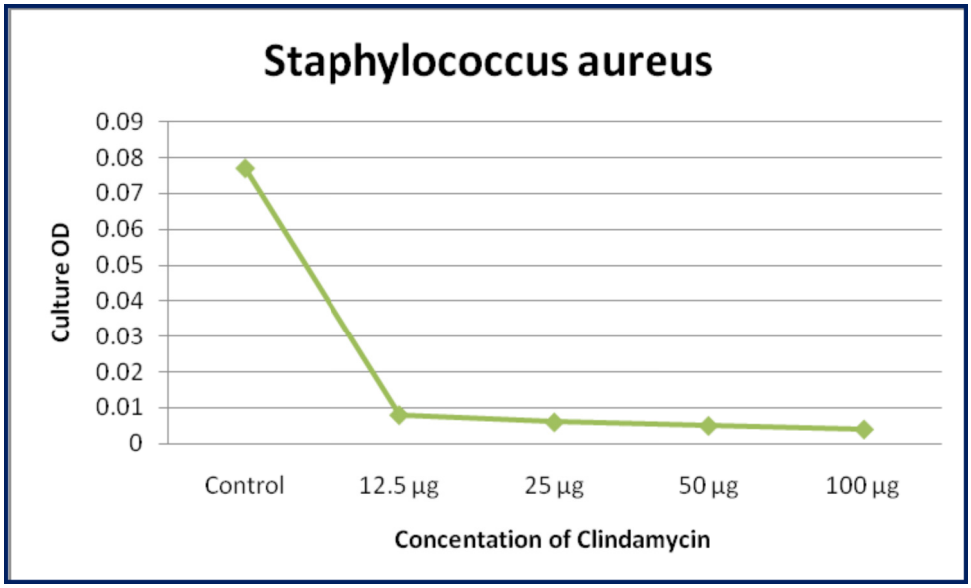


Figure 7.36: MIC of Clindamycin implant in *Staphylococcus aureus*

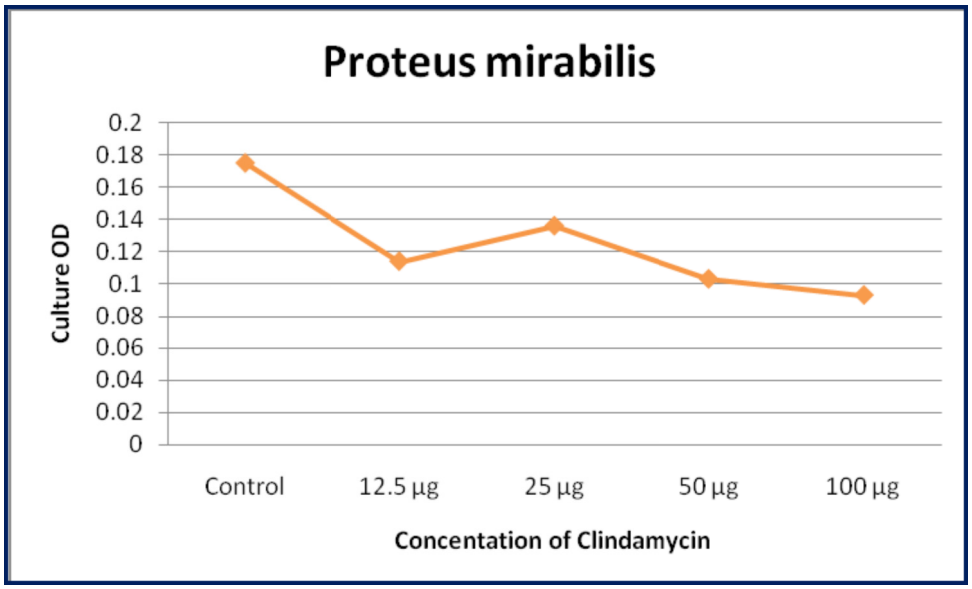


Figure 7.37: MIC of Clindamycin implant in *Proteus mirabilis*

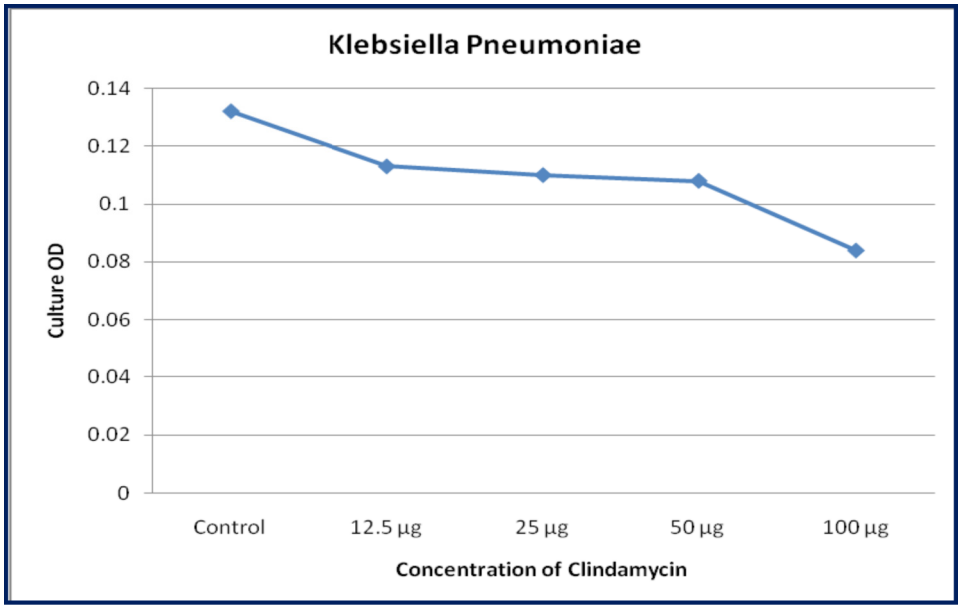


Figure 7.38: MIC of Clindamycin implant in *Klebsiella pneumoniae*

7.7.2 Disc diffusion and agar well diffusion method

The diameter of zone of inhibition was measured and the value was shown in the table 7.26.

Table 7.26: Diameter of zone of inhibition of Clindamycin implants

ORGANISM NAME	AGAR WELL DIFFUSION METHOD (ZONE SIZE)					DISC DIFFUSION METHOD (ZONE SIZE)			
	12.5µg	**25µg**	**50 µg**	**100 µg**	**200 µg**	**+ive Control (2 µg)**	**2 µg**	**4 µg**	**16 µg**
Staphylococcus aureus	6 mm	9mm	11mm	13mm	17 mm	5 mm	6 mm	7mm	11mm
Enterobacter aerogens	4 mm	5mm	7 mm	10 mm	16 mm	1 mm	1.5mm	3 mm	5 mm

+ive control = Standard Clindamycin disc

After 24hrs of incubation, the diameter of zone of inhibition was measured and it was compared with the standard drug diameter. It shows better antibacterial activity over those micro-organisms. The zone of inhibition of Clindamycin implant by agar disc diffusion and agar well diffusion method was shown in the following figures.

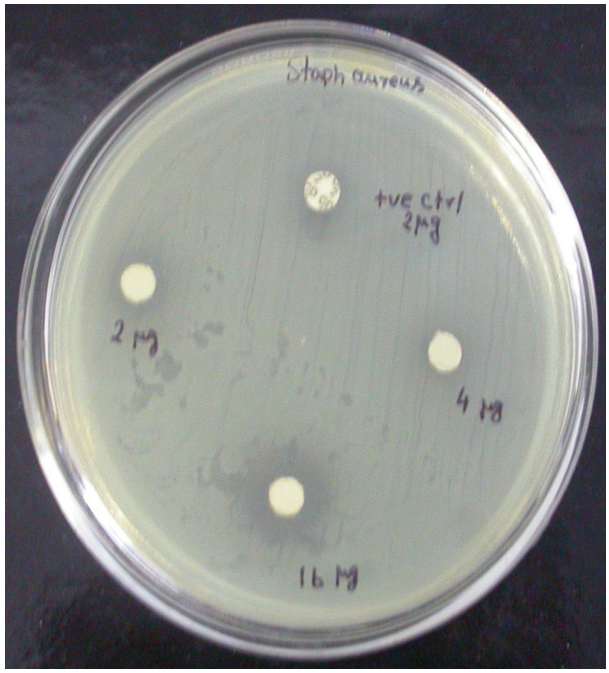


Figure 7.39: Disc diffusion zone for *Staphylococcus aureus* using Clindamycin implant pad

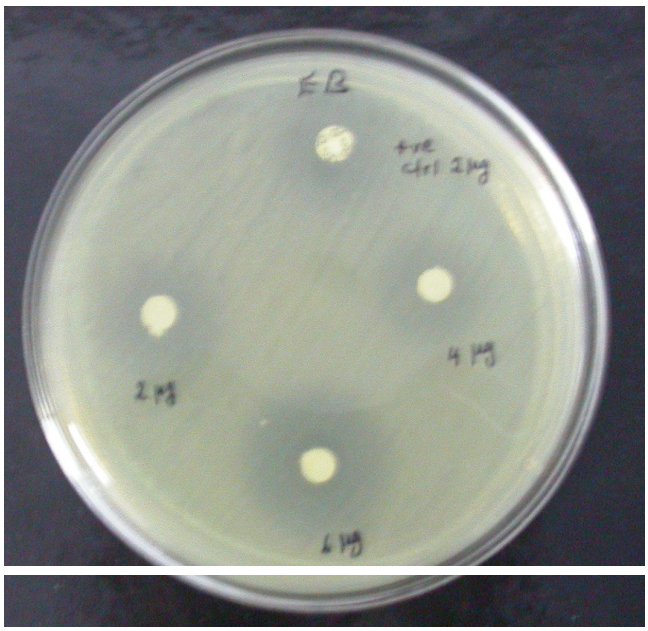


Figure 7.40: Disc diffusion zone for *Enterobacter aerogens* using Clindamycin implant pad

Figure 7.41: Agar well diffusion method in *Staphylococcus aureus* using Clindamycin liquid

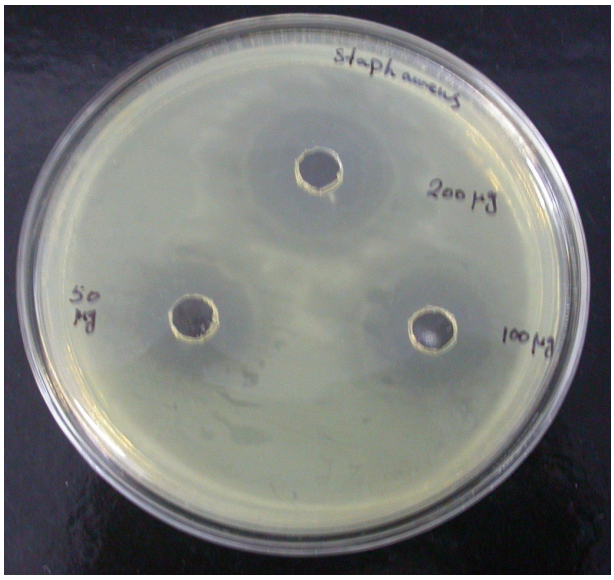


Figure 7.42: Agar well diffusion method in *Staphylococcus aureus* using Clindamycin liquid

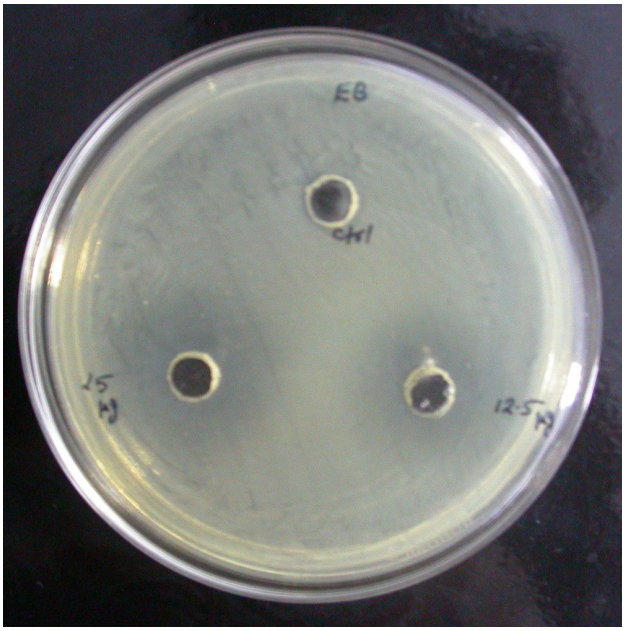


Figure 7.43: Agar well diffusion method in *Enterobacter aerogens* using Clindamycin liquid

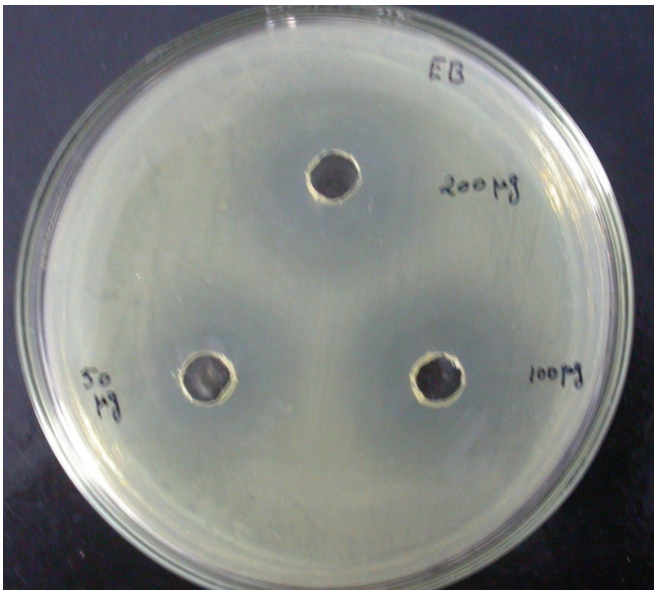


Figure 7.44: Agar well diffusion method in *Enterobacter aerogens* using Clindamycin liquid

8. SUMMARY AND CONCLUSION

Development of implantable drug delivery system is triggered by the need to control the drug release and for the localized therapy.

Clindamycin hydrochloride was incorporated in gelatin and sodium alginate polymer in different concentrations. The various physicochemical parameters, drug release studies and antimicrobial activity were performed for the developed implant.

The following conclusions were drawn from the results obtained.

- In the preliminary screening, the drug undergo melting point determination, IR analysis and UVscanning, the result obtained was complied with the USP standards.
- From the FT-IR spectra, it was observed that similar characteristic was observed that similar peaks appear for the drug and their formulation. Hence, it shows that there was no chemical interaction between the drug and polymer used.
- The DSC thermograms obtained for the pure drug and for the formulation showed no significant shift in the endothermic peaks confirming the stability of the drug in the formulation.
- From the results of the weight uniformity and thickness, it can be inferred that all the formulations exhibited uniform weight and thickness with low standard deviation values.
- All the formulations were found to obtain uniform quantity of drug as per content uniformity studies indicating reproducibility of the technique.
- For all the formulation, the percentage moisture showed maximum loss except F7 because of low concentration of gelatin undergo moisture loss in dry condition.
- From the result of invitro release studies of the formulation F1 to F9, the formulation F7 showed an sustained release at the end of 24^{th} hour due to the high concentration of gelatin and sodium alginate, 700 and 300 mg respectively.
- The ‘n’ values of various mathematical model fittings suggests that all the films exhibit anamolous transport. It had concluded that the prepared implant undergo diffusion mechanism
- The antimicrobial activities were performed on *Staphylococcus aureus* and *Enterobacter aerogens*, the zone of inhibition was observed by agar disc diffusion and agar well diffusion method. From the results, the optimized implant shows better activity over those micro-organisms.
- The result of stability studies carried out on optimized formulation indicate that after one month at room temperature, dark place and direct sunlight. The drug content

reduced markedly after exposing sunlight and there was no significant change at room temperature and dark place.

- The future plan is to carry out the invitro-invivo correlation studies.

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